UNIVERSITY OF LIVERPOOL

The Synthesis of Chiral *N*-Oxides and Their Applications to β-Turn Mimetic Design

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

by

Seán P. Richards

January 2019

Declaration

This thesis is the result of my own work. The material contained in this thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or qualification.

Seán P. Richards

The research was carried out in the Department of Chemistry, University of Liverpool and LifeArc, SBC open innovation campus, Stevenage.

Abstract

Chapter 1 gives a brief introduction to tertiary amine *N*-oxides and enamine *N*-oxides as functional groups, including the properties, synthesis, reactions of and applications of these types of compounds. An overview of amino acid and protein structure is also covered, as well as how these structures are used in the design of *β*-turn mimetics as therapeutics.

Chapter 2 begins by outlining the work carried out within the O'Neil research group towards the synthesis of chiral, fused morpholine *N*-oxides. Also introduced is the application of these bicyclic derivatives to an active protein-protein interaction (PPI) project being carried out by our industrial sponsors LifeArc. The synthesis of a series of *β*-turn mimetics based on these morpholine systems, as well as their application to the PPI project is described. Parallel to this work, the synthesis of a variety of chiral, functionalised bicyclic enamine *N*-oxides by the tandem Cope elimination/reverse-Cope cyclisation has been carried out.

Chapter 3 starts with a summary of the diastereoselective oxidation of proline derivatives to give homochiral *N*-oxide, which has been carried out within the O'Neil group. The synthesis of further functionalised *N*-benzyl proline derivatives, as well as the diastereoselective oxidation of these tertiary amine species is described. Oxidation of analogues with no hydrogen bond donor present in the pyrrolidine side chain is also discussed, in order to examine the underlying reasons for the diastereoselective of oxidation observed. Additionally, the synthesis of a series of chiral, functionalised bis-*N*-oxides based on these tertiary amine systems is outlined.

Chapter 4 gives the experimental details and analytical data of all compounds described in Chapters 2 and 3.

Acknowledgements

I would like to thank my supervisor Dr Ian O'Neil for the opportunity to work for him over the last few years. All of the help, guidance and top quality vino was greatly appreciated. Fingers crossed without me out of the way (only just) the trips to the Royal will be less frequent. All jokes aside, it's been a pleasure.

I would also like to thank my industrial sponsors LifeArc for the funding to carry out this work. As well as my industrial supervisor Dr Jon Large for all of the check-ins and great advice when called upon. Not forgetting of course thank you to the whole team down in Stevenage; Lisa H, Joel B and Chido M for their input into these projects and Caitlyn and Emily, for making Stevo bearable for the summer spent down there.

A special mention for Dr Neil Kershaw. Where do I start? Without the impromptu chemistry lessons, answering my calls of "small fire!" or just giving me a kick up the arse when I needed it I wouldn't have made the progress that I have in the last few years. All the best.

Thank you to all of the members of the 4th floor both past and present, there're too many of you to mention but a few honourable mentions should be given: Gina W, Emma S, Paul M, Matt P, Chris W, Rachel C, Nathan C, Sarah L and Ryan M. Oh, and I guess Mike R as well if I have to. Thank you all for the AJ trips, group outings and just generally putting up with me day to day, I know it's been a struggle.

A quick word for Konstantin, another regular of the mid-week AJ pilgrimage, and the analytical services for all the help over the past 4 years.

Last but by no means least I would like to say thank you to by Mum and Dad. Cheers for all of the support and encouragement, I certainly needed it, I couldn't have done it without you both.

Abbreviations

AA	Amino acid
Å	Ångström
AIBN	Azobisisobutyronitrile
aq.	Aqueous
Boc	*tert*-butoxycarbonyl
COSY	Correlated spectroscopy
CI	Chemical ionisation
EDG	Electron donating group
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
ee.	Enantiomeric excess
eq.	Equivalent*(S)*
ESI	Electrospray Ionisation
EWG	Electron withdrawing group
HOBt	Hydroxybenzotriazole
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
IR	Infra-red
LC-MS	Liquid chromatography-mass spectroscopy
m-	meta
M	Molar
m-CPBA	*meta*-chloroperoxybenzoic acid

mmol	Millimole*(S)*
mol	Mole*(S)*
MW	Molecular weight
NaHMDS	sodium bis(trimethylsilyl)amide
NMO	*N*-methyl morpholine *N*-oxide
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
o/n	Overnight
p-	para
PPI	Protein-protein interaction
ppm	Parts per million
rt	room temperature
RU	Resonance units
SPR	Surface plasmon resonance
TBAI	Tetrabutylammonium iodide
TBDMS	*t*-butyldimethylsilyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMAO	Trimethylamine *N*-oxide
TPAP	Tetrapropylammonium perruthenate
VDW	Van Der Waals

Contents

Chapter 1

Introduction

1. Introduction

The work carried out in this thesis involves the synthesis of tertiary amine *N*-oxides, also termed "amine oxides", by a variety of methods. The dative N^{+}—O^{-} bond is the distinguishing feature of *N*-oxide species. This section aims to give a background to the characteristics, synthesis and applications of this family of compounds.

1.1. Tertiary Amine Oxides

1.1.1. Discovery, Structure and Properties

The existence of tertiary amine oxides was first reported at the end of the 19th century by Pinner and Wolffenstein.[1] However, the exact structure was not determined until 1939 when the first X-ray crystal structure of a tertiary amine oxide, trimethylamine oxide, was published by Lister and Sutton.[2] This showed that tertiary amine oxides were species in which a sp^3 hybridized nitrogen atom is datively bonded to an oxygen atom as well as three alkyl or aryl substituents (Figure 1.1).

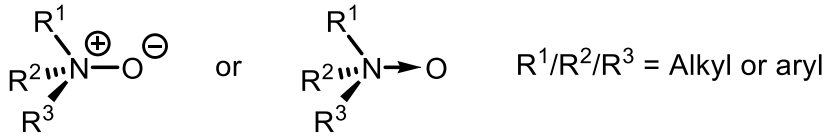


Figure 1.1: General structure of a tertiary amine *N*-oxide.

This initial crystal structure of TMAO and subsequent X-ray studies of other tertiary amine oxides, showed that the intrinsic N-O bond has a distance between 1.36-1.41 Å.[2-5] These studies also revealed the tetrahedral arrangement about the central nitrogen, creating a stereogenic centre if $R^1 \neq R^2 \neq R^3$.

The highly polar nature of the N-O bond, with dipole moments in the region of 4.5-5.0 Debyes, is central to the physico-chemical properties of these compounds.[6] Tertiary amine oxide species are slightly basic, however they are substantially less so than their parent amine counterparts. They can behave as Brönsted bases and when combined with acids will form their corresponding hydroxyammonium salts, which have pK_as = 4-5 (Figure 1.2).[7]

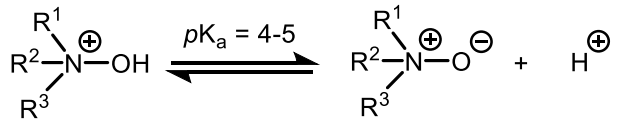


Figure 1.2

Tertiary amine oxides can be stabilised by intermolecular hydrogen bonding to water, alcohols or acids.[8-10] Alternatively, stabilisation by intramolecular hydrogen bonding can occur between the *N*-oxide and one or more hydrogen bond donor groups such as alcohols, carboxylic acids and amides.[11, 12]

Due to this high H-bonding ability tertiary amine oxides are commonly soluble in alcohols and polar aprotic solvent, with limited solubility in non-polar organic solvents.[13] They can also be very hygroscopic and are often isolated in their hydrated form.[14]

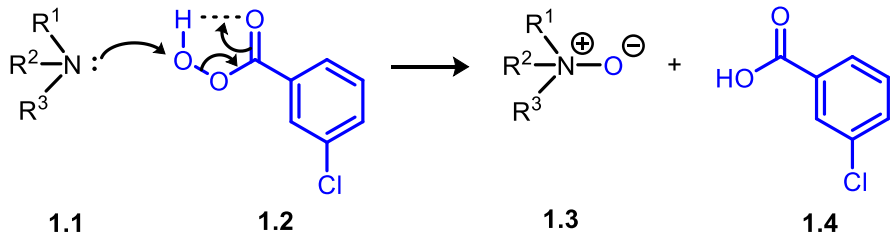
1.1.2. Synthesis of Tertiary Amine *N*-Oxides

1.1.2.1. Direct Oxidation of Tertiary Amines

There are a number of methods for the synthesis of tertiary amine *N*-oxides. By far the most widely utilised is the direct oxidation of the parent tertiary amine compound. A wide variety of reagents have been used to carry out this transformation including: hydrogen peroxide[15, 16], alkyl/biomimetic hydroperoxides[17, 18], peracids[19, 20], molecular oxygen[21], ozone[22], oxaziridines[23] and selenoxides[24] amongst others.

Traditionally, hydrogen peroxide in water or alcoholic solutions has been used for the oxidisation of tertiary amines to their corresponding *N*-oxides. However, this method is commonly low yielding and requires further purification steps.[25, 26] First described by Craig and Purushothaman in 1970 oxidation with *m*-CPBA, a peroxycarboxylic acid, has being utilised as the primary method of direct tertiary amine oxidation in recent years.[19] This will also be the primary method for direct oxidation of tertiary amines in this thesis. *m*-CPBA **1.2** oxidises tertiary amines **1.1** in a concerted pathway, as shown in Scheme 1.1, to form the tertiary amine oxide **1.3**, with *meta*-chlorobenzoic acid **1.4** as the only by-product. The mild reaction conditions, often at or below room temperature as well as the relatively quick reaction time drives the preference for *m*-CPBA as the oxidant of choice in most of academic scale research.[27] However,

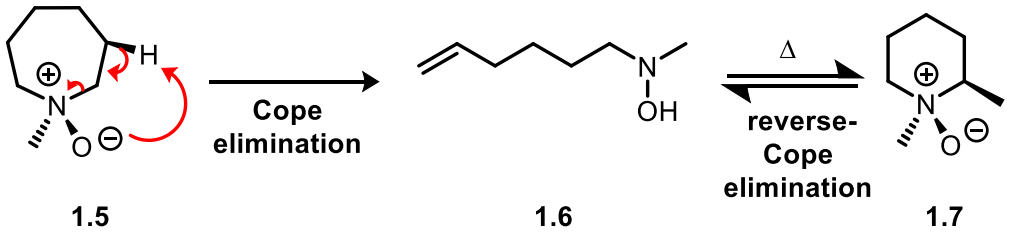
industrially *m*-CPBA is used far less frequently utilised due to the stoichiometric quantities of oxidant required and the potential of *m*-CPBA to be explosive at >85% purity.[28]



Scheme 1.1: General mechanism of *m*-CPBA oxidation of tertiary amines.

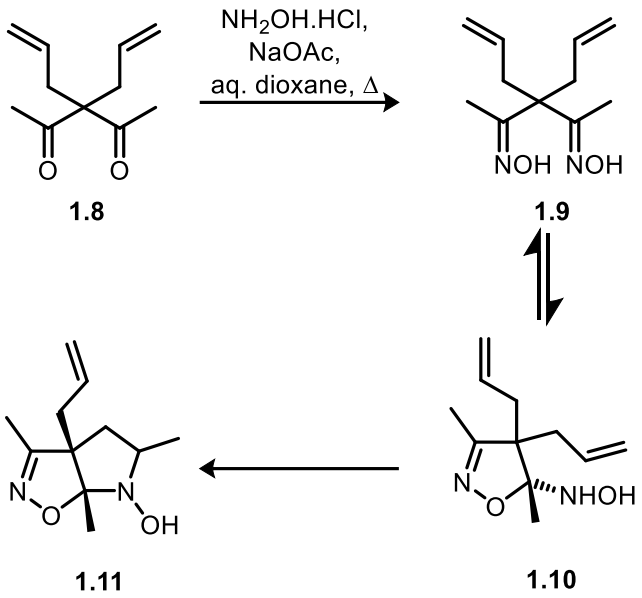
1.1.2.2. The Reverse-Cope Cyclisation

An alternative method of amine *N*-oxide synthesis is the reverse-Cope cyclisation. As the name suggests this reaction is the reverse of the classical Cope elimination process and proceeds through a concerted pathway, leading to a cyclised product. The first report of this reaction was made by Cope and LeBel in 1959. Whilst investigating the Cope elimination of *N*-oxide **1.5** to the corresponding hydroxylamine adduct **1.6** they unwittingly observed the reverse Cope cyclisation product **1.7**, as shown in Scheme 1.2.[29]



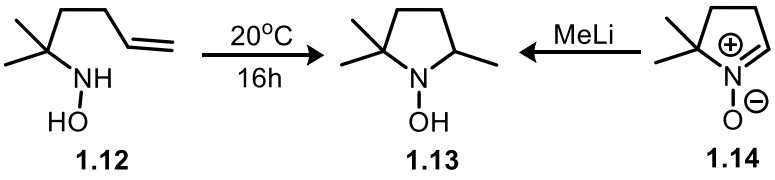
Scheme 1.2: First observed reverse-Cope elimination reaction

The reaction was not formerly reported until 1976 by House and co-workers, when they observed an annulated pyrrolidine **1.11** as opposed to the desired dioxime **1.9** in the reaction of 1,3-dione **1.8** and hydroxylamine hydrochloride under basic conditions (Scheme 1.3).[30] They postulated that the unexpected product had formed by the intramolecular reverse-Cope cyclisation of the hydroxylamine **1.10**, which existed in equilibrium with the initially anticipated dioxime **1.9**.



Scheme 1.3: Reverse-Cope cyclisation reported by House and co-workers.[30]

House and Lee later confirmed their suspicions with experiments on simpler, model systems, where again they observed a reverse-Cope cyclisation product **1.13** when the allyl hydroxylamine **1.12** was allowed to stir for 16 hours at 20 °C (Scheme 1.4).[31] To confirm the structure of the hydroxylamine **1.13** it was synthesised *via* an unambiguous route, where **1.14** was treated with methyllithium causing alkylation to afford hydroxylamine **1.13**, which by comparing spectra was shown to be the same species as isolated from the reverse-Cope cyclisation reaction.

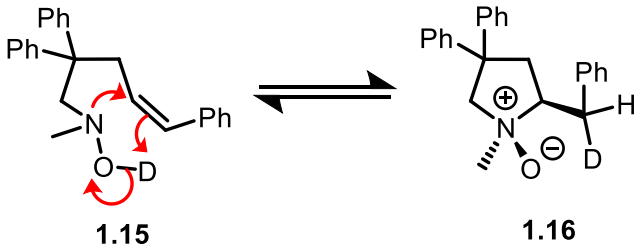


Scheme 1.4: House and Lee reverse-Cope cyclisation confirming experiments.[31]

House had initially proposed a radical process *via* a nitroxide intermediate for the mechanism of the cyclisation. However, work by Black and Doyle disproved this theory by the introduction of radical inhibitors to the reaction, which had no effect on the rate of reaction.[32]

The mechanism of the reverse Cope elimination was not correctly verified until 1990 by Ciganek who used deuterium labelled hydroxylamines to understand the reaction pathway of the cyclisation (Scheme 1.5).[8] He concluded that the reaction took place

by a concerted *syn* process, *via* a five-membered transition state, resulting in the *cis* product **1.16**. He also showed, that under suitable conditions, the reaction was reversible. These observations were later confirmed by Oppolozer and co-workers during their work on the cyclisation of *(E)* and *(Z)*-alkenyl hydroxylamines.[33]



Scheme 1.5: General mechanism of the reverse-Cope cyclisation.

Following this key initial discovery subsequent publications from Ciganek led to a more complete understanding of the intricacies of the reaction. Due to the reversible nature of the reverse-Cope cyclisation, synthesis of strained 3- and 4-membered rings is not possible, instead favouring the hydroxylamine form. Cyclisations to 5- and 6-membered rings are favourable, whilst formation of 7-membered rings is disfavoured, rather remaining in their hydroxylamine form (Figure 1.3).[34, 35]

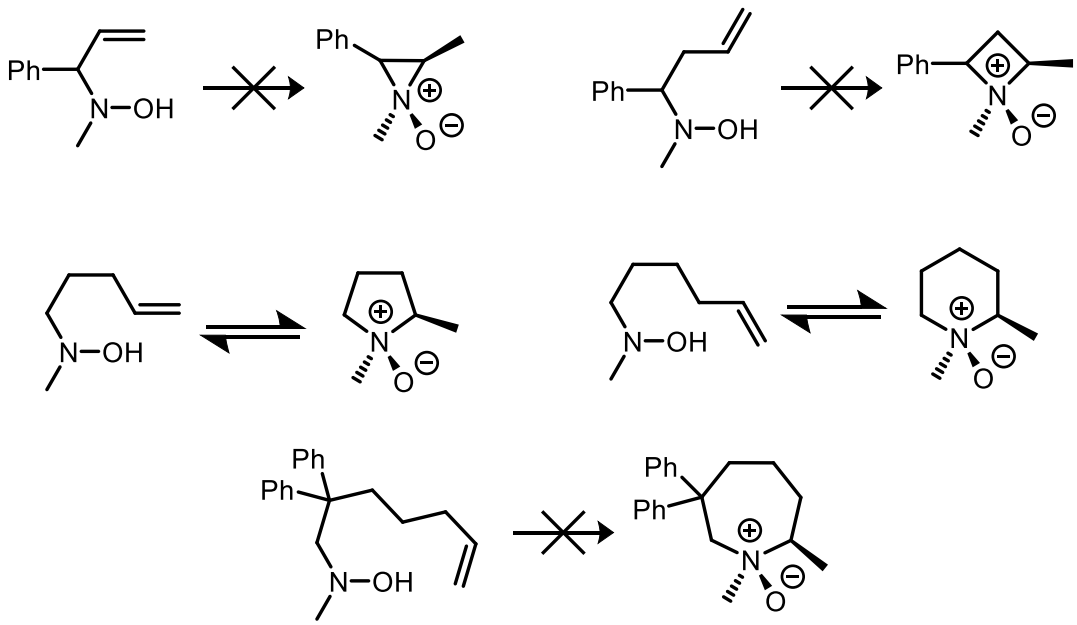


Figure 1.3: Favoured and disfavoured reverse-Cope cyclisation reactions.

These reactions were also shown to be strongly solvent dependent, with polar, hydrogen bonding solvents favouring the reverse Cope elimination product and non-polar solvents favouring the hydroxylamine starting materials.[35]

A number of later publications have delved deeper into other factors effecting this reaction, including substituent effects on the alkene and this is summarised in Figure 1.4. These effects and others are summarised in a 2004 review by Knight and Cooper.[36]

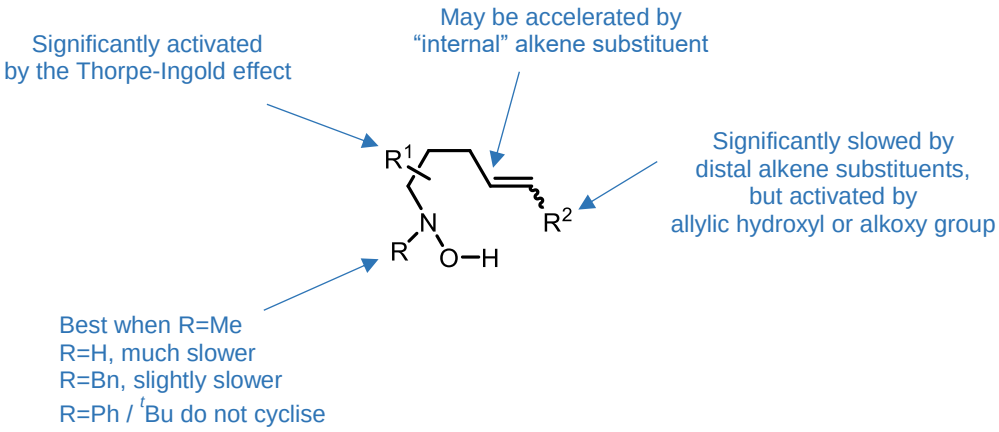
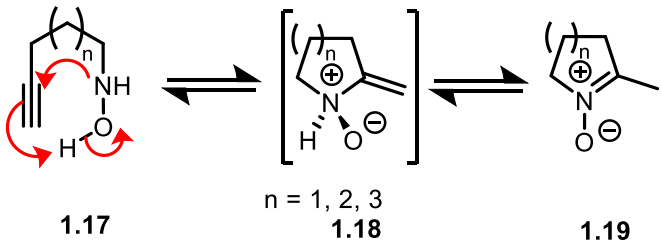


Figure 1.4: Summary of substituent effects on the reverse-Cope cyclisation reaction by Knight and Cooper.

Reverse Cope cyclisations have also been carried out using alkynes as the substrate instead of alkenes. The mechanism of cyclisation remains the same, however the two vary in a few respects. In the case of alkyne substituents, following initial reverse Cope elimination, a proton transfer from the initially formed enamine *N*-oxide species **1.18** to the more stable cyclic nitrone **1.19** takes place (Scheme 1.6).[37]



Scheme 1.6: Reverse-Cope cyclisation of alkyne derivatives.

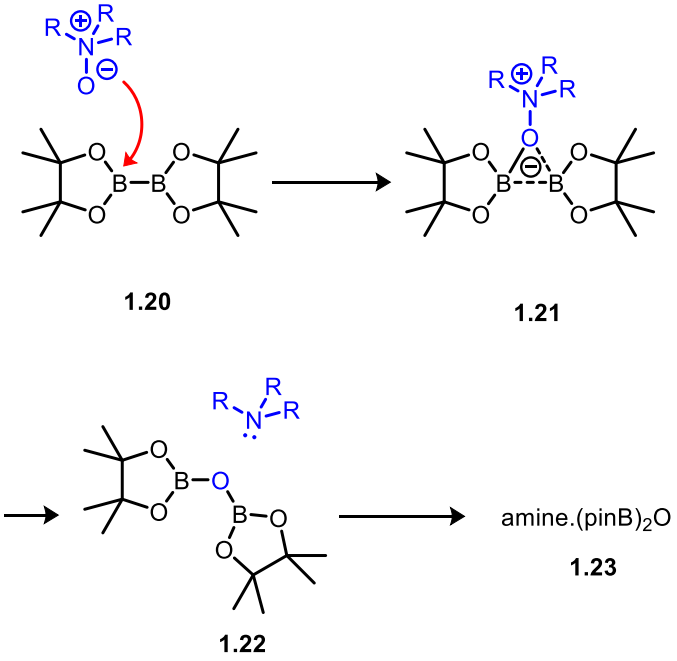
Reverse Cope eliminations with alkynes also allow for formation of 7-membered rings, which was not tolerated with alkene derivatives.[38] A wider variety of distal substituents have also been shown to be accepted for the alkyne reverse Cope cyclisations, including silyl and alkoxyl groups.[39, 40]

One final method of tertiary amine *N*-oxide formation of note is the alkylation of hydroxylamines with alkyl halides, first reported by Dunstan and Goulding in 1899.[41] However, this method has not been utilised in any of the work described in this thesis, therefore will not be discussed further.

1.1.3. Reactions of Tertiary Amine N-Oxides

1.1.3.1. *N*-Oxide Reduction

Tertiary amine oxides can undergo a number of synthetically useful transformations. The simplest of these transformations is the reduction of the *N*-oxide species to the parent amine, which is of particular use when the *N*-oxide has been employed as an amine protecting group. There are a number of ways to carry out this reaction, such as treatment with bakers' yeast[42], sulfur reagents[43, 44], phosphorus reagents[45, 46], $LiAlH_4$[17] and metal catalysed hydrogenation[47]. One limitation of the majority of these methods is that many other functional groups undergo reduction using these reagents. Lakshman and co-workers have developed a facile reduction method of tertiary amine oxides utilising the diboron reagent $(pinB)_2$ **1.20**, shown in Scheme 1.7**.** The mild reagent and conditions, and straightforward product isolation, allows for selective reduction of the tertiary amine oxide in the presence of a wide range of other functionalities, such as alkenes and alkynes *via* the proposed mechanism below (Figure 1.7).[48]



Scheme 1.7: *N*-Oxide reduction using by $(pinB)_2$.

1.1.3.2. The Meisenheimer Rearrangement

The Meisenheimer rearrangements, first described in 1919 are a thermally driven, sigmatropic rearrangement of tertiary amine *N*-oxides to *O*-allyl hydroxylamines, or hydroxylamines if R^3=H.[49] This rearrangement can take place *via* two distinct mechanisms depending on the structural makeup of the initial tertiary amine oxide (Figure 1.5).

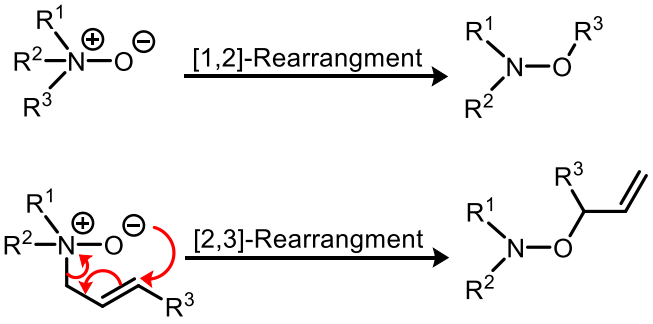
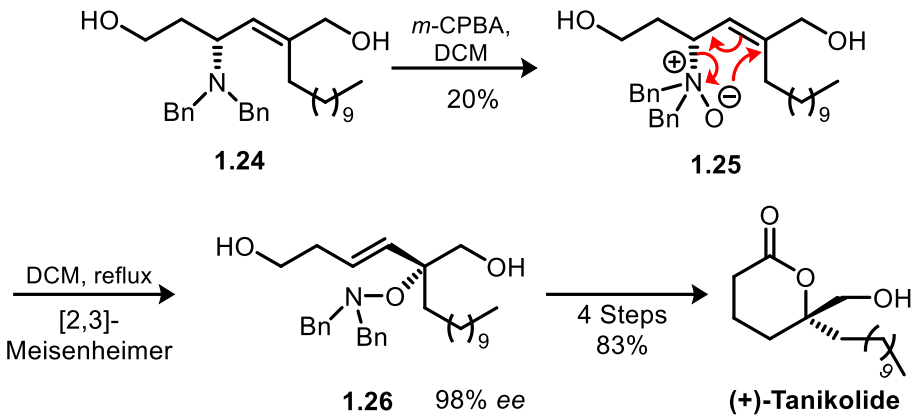


Figure 1.5: Variations of the Meisenheimer rearrangement.

A [1,2]-rearrangement can take place by a radical mechanism to give the hydroxylamine species, but only when the migrating group can form a stabilised radical (eg. benzyl).[50] Alternatively, with an allyl substituted *N*-oxide, a concerted [2,3]-sigmatropic rearrangement will take place to give the *O*-allyl hydroxylamine. If a suitably positioned *β*-hydrogen is present within the *N*-oxide then there can be a competing Cope elimination process also taking place.

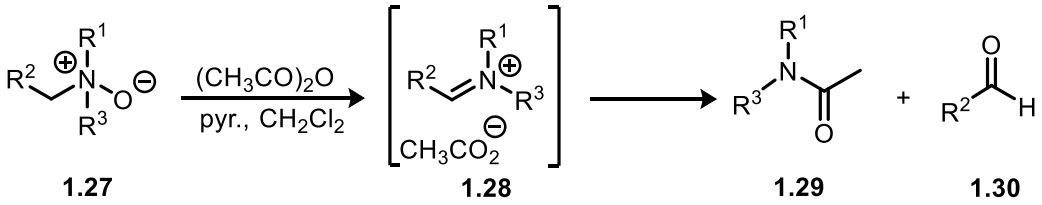
If suitably set up, the Meisenheimer rearrangement will proceed in a stereospecific manner, which has been applied in the total synthesis of a number of compounds such as magallanesine, (-)-flueggine, (+)-virosaine and (+)-tanikolide, the last of which is shown in Scheme 1.6.[51-53] (+)-Tanikolide was synthesised by Yang and co-workers, who showed that showed that oxidation of tertiary amine **1.24** with *m*-CPBA gave the *N*-oxide **1.25**, which underwent a [2,3]-Meisenheimer rearrangement when heated in DCM to afford the chiral alcohol **1.26** in 98% *ee*.. Three subsequent steps, which maintained the stereochemistry introduced by the stereospecific Meisenheimer rearrangement, yielded the pure (+)-tanikolide in an 83% yield.



Scheme 1.6: Stereoselective Meisenheimer rearrangement in the total synthesis of (+)-Tanikolide.

1.1.3.3. The Polonovski Reaction

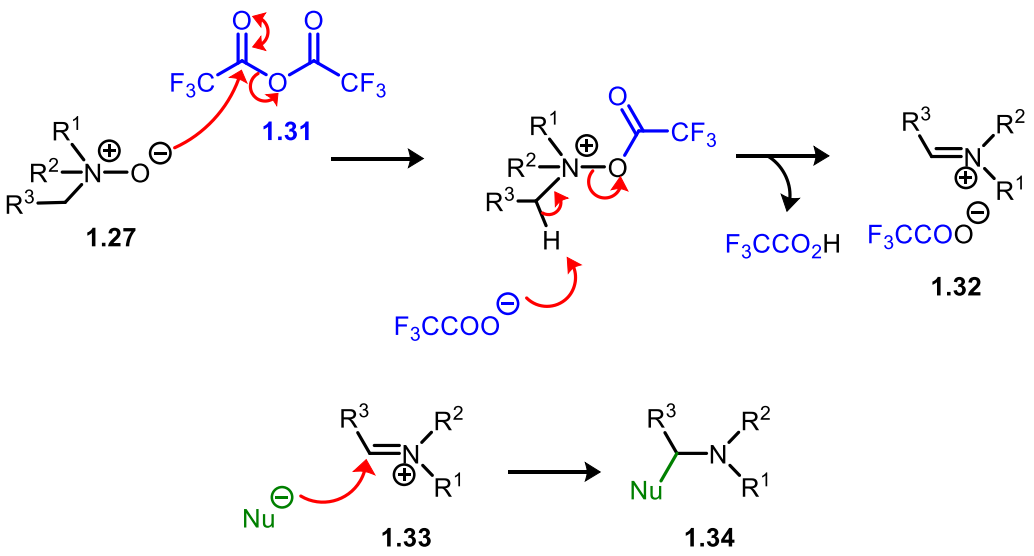
The Polonovski reaction, first reported in 1927, is a thermodynamically driven rearrangement of an *N*-oxide to give an *N*,*N*-disubstituted acetamide **1.29** and an aldehyde **1.30**, upon treatment of the *N*-oxide **1.27** with acetic anhydride.[54] The reaction, which is shown in Scheme 1.7, proceeds *via* an iminium ion intermediate **1.28**. Under the harsh conditions required for the Polonovski reaction, often heating to well over 100 °C, the iminium ion intermediate **1.28** is not stable and will react immediately with the acetate ion present.



Scheme 1.7: General reaction scheme of the Polonovski reaction

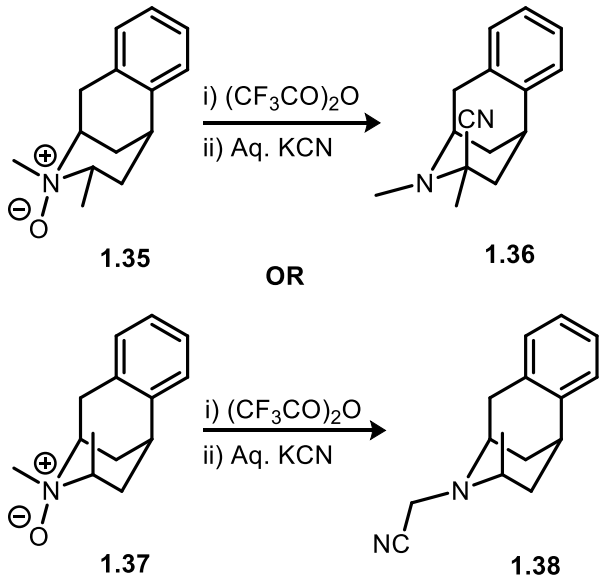
A variation of this reactions, called the Polonovski-Potier reaction, replaces acetic anhydride with TFAA **1.31**, which allows for the reaction to be carried out under much milder conditions so it can be stopped at the iminium ion stage.[55-57] Having the ability to do this means that the iminium ion species **1.32** can be trapped out with

nucleophiles, an example of this is shown in the mechanism in Scheme 1.8, to give the α-functionalised amine **1.34**.



Scheme 1.8: General mechanism of the Polonovski-Potier reaction

This method was applied in the synthesis of the α-aminonitriles **1.36** and **1.38**, shown in Scheme 1.9. *N*-Oxides **1.35** and **1.37** were treated with TFAA to produce the iminium ion intermediates, which were then quenched with aqueous KCN to yield the α-aminonitriles.



Scheme 1.9: Regioselective Polonovski-Potier reaction to synthesise α-aminonitriles **1.36** and **1.38**.

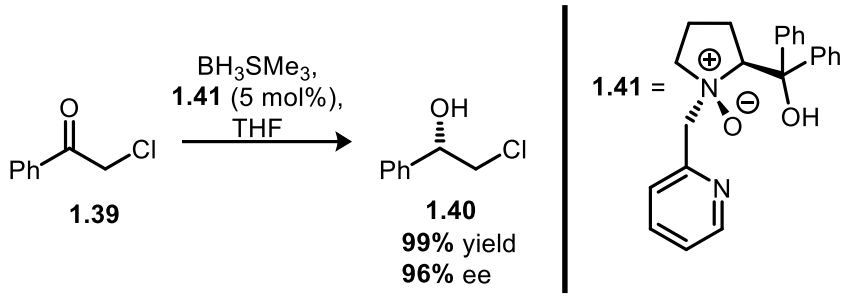
Interestingly, the stereochemistry of the *N*-oxide starting materials affected the regioselectivity of the reaction, *β*-elimination to give the iminium ion species must remove an axial proton, of which there is one on the ring of **1.35** but not in **1.37**. Therefore **1.35** reacts *via* the more stable iminium ion intermediate within the ring to yield **1.36**, whereas **1.37** reacts *via* a terminal iminium ion to afford the *α*-aminonitrile **1.38**.[58]

1.1.4. Tertiary Amine *N*-Oxides in Catalysis

Tertiary amine *N*-oxides have been utilised in a number of ways synthetically in the area of catalysis. More traditionally they have been employed as co-oxidants, to regenerate the catalyst used in the transformation. An example of this is the Upjohn dihydroxylation of alkenes. This method uses NMO as the co-oxidant, with catalytic amounts of the toxic and expensive osmium tetroxide, for the *cis*-dihydroxylation of a wide variety of alkenes.[59, 60] NMO has also been employed as the co-oxidant in the Ley-Griffith oxidation using TPAP, a ruthenium based catalyst, for the oxidation of primary and secondary alcohols to aldehydes and ketones respectively.[61, 62]

More recently focus has turned to the use of chiral tertiary amine *N*-oxides themselves as the catalysts, either as organocatalyts or metal binding ligands in asymmetric transformations. In both cases the nucleophilic character of the *N*-oxide oxygen is used as a driving force for their binding ability to either substrate or a metal centre.

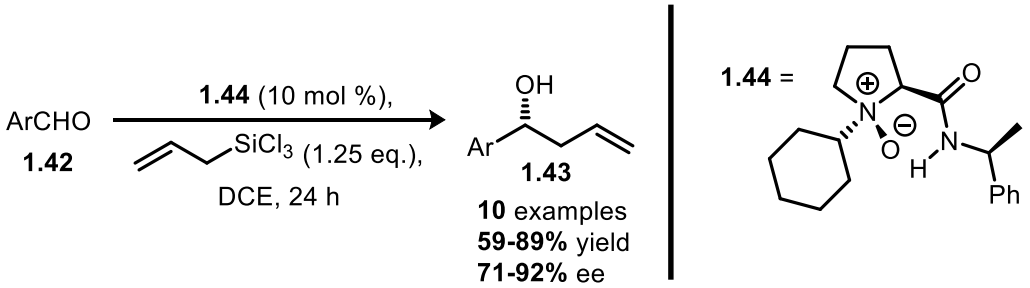
Some early work in the area was carried out within the O'Neil group, exhibiting the **first example** of using proline derived chiral tertiary amine oxide **1.41** to catalyse the enantioselective borane reduction of ketones **1.39** (Figure 1.10).[63] It was established that the presence of a heteroatom in the aryl group on the amine, as well as the added steric bulk of the diphenyl groups, increased enantioselectivity.



Scheme 1.10: Enantioselective borane reduction of ketones catalysed by a chiral tertiary amine *N*-oxide **1.41**.

These findings were backed up further by Feng and co-workers, when the same amine *N*-oxide species was used as a complex with titanium (IV) isopropoxide for the asymmetric cyanosilylation of ketones, this time giving the products in a moderate 69% *ee*.[20]

The pyrrolidine side chain functionality of the chiral amine oxide catalysts has also been investigated as a way to elicit more control of reactions. Amides have been shown to enhance the stereoselectivity by offering an additional co-ordination point, in the form of the carbonyl oxygen, as well as diverse chain options by simple amide coupling synthesis. A good example of this is shown in the enantioselective allylation of a variety of aromatic aldehydes **1.42** in work done by Snapper and co-workers catalysed by the chiral *N*-oxide **1.44** (Figure 1.11).[64]



Scheme 1.11: Allylation of aromatic aldehydes catalysed by tertiary amine *N*-oxide **1.44**.

Based on this initial work, Feng and co-workers have carried out extensive research in the area of chiral amine oxides as asymmetric catalysts. This has focussed mainly on the development of C_2-symmetric *N,N'*-dioxides for use as organocatalysts or metal ligands in asymmetric catalysis, the general structures of which are shown in Figure 1.6.[65]

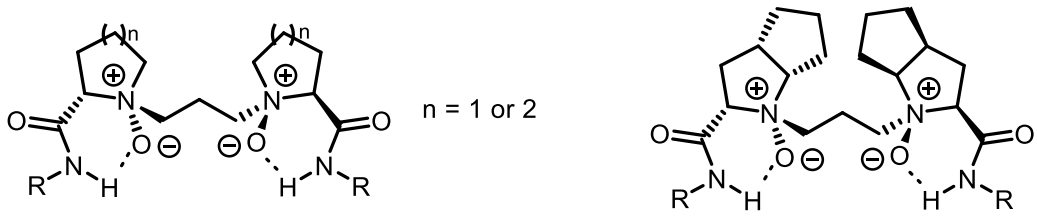
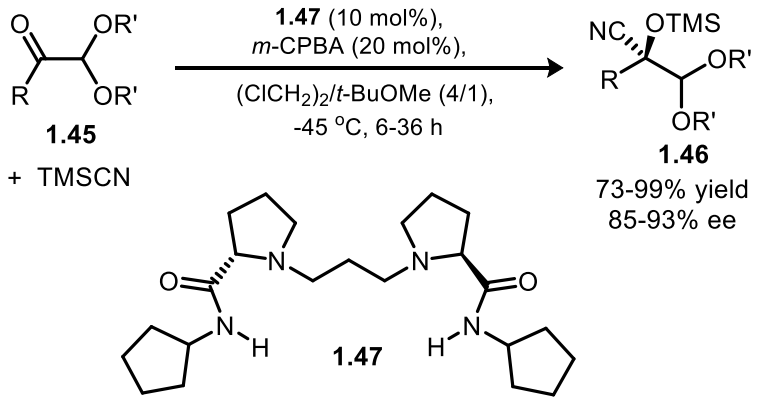


Figure 1.6: Feng and co-workers C_2-symmetric *N,N'*-dioxide catalysts.

By exploiting the high nucleophilicity of the *N*-oxide oxygen, along with the high affinity of silicon for oxygen, these *N,N'*-dioxides have been shown to activate organosilicon reagents in the silylcyanation of aldehydes, ketones (Figure 1.12) and imines in the absence of any metal.[66-68] Additionally, the catalyst can be formed *in situ* by heating the parent diamine **1.47** with 0.2 eq. of *m*-CPBA to oxidise to the *N,N*-dioxide species.



Scheme 1.12: Ketone silylcyanation reactions catalysed by *N,N'*-dioxide **1.47**.

These *N,N'*-dioxide species have also been shown to co-ordinate well to a wide variety of metal centres such as Cu (I)[69], In(III)[70], Sc (III)[71] and Ni (II)[72] amongst others to catalyse a diverse range of asymmetric transformations. The ability of the *N*-oxide to bind to the metal centre is essential for their success as chiral catalysts.

All these examples of chiral amine *N*-oxides being used as homochiral catalysts share the same method of preparation, namely the direct oxidation with *m*-CPBA either as a final synthesis step or *in situ*, to give the *N*-oxide or *N,N'*-dioxide species as single diastereoisomers in all cases. The *syn* diastereoselectivity of these types of oxidations has been accepted for some time now, however little work has been done to explore this. This along with probing the scope for pyrrolidine side chain functionality will be discussed in detail in Chapter 3.

1.2. Enamine *N*-Oxides

1.2.1. Structure and Properties of Enamine *N*-Oxides

Enamine *N*-oxides are by far the least well reported of the *N*-oxide family. This functional group consists of a quaternary, positively charged nitrogen datively covalently bonded to an oxygen with two alkyl substituents and one vinylic group (Figure 1.7). Compared to the high level of understanding about their tertiary amine counterparts, very little is known about the properties, synthesis and reactions of enamine *N*-oxides.

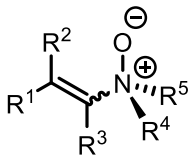


Figure 1.7: General structure of an enamine *N*-oxide.

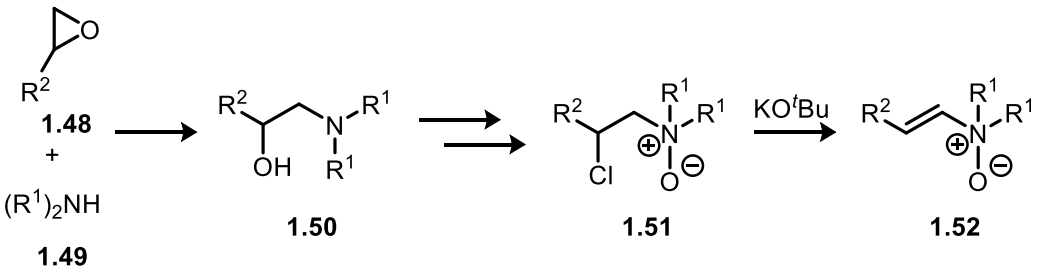
Like tertiary amine *N*-oxides, enamine *N*-oxides are highly polar, hygroscopic compounds. It has also been anticipated that they may also possess good metal binding properties, which could make them interesting compounds for ligand design. It is this potential use, as well as interest in learning more about the chemical properties of this functional group that drives research in this area.

1.2.2. Synthesis of Enamine *N*-Oxides

Work towards a deeper understanding of enamine *N*-oxides has been hampered predominantly by the difficulty in their synthesis. Simple *N*-oxidation of the parent enamine is not an option, as treatment of this with oxidising agents leads to the oxidation of the highly electron rich enamine double bond and subsequent rearrangements to give undesired products.[73-75]

The first known enamine *N*-oxides were first prepared by Winterfeldt and Krohn in 1969 as transient intermediates in cycloadditions.[76] This was later confirmed by NMR studies done by Hwu and co-workers in 1993 during work on an indole synthesis.[77]

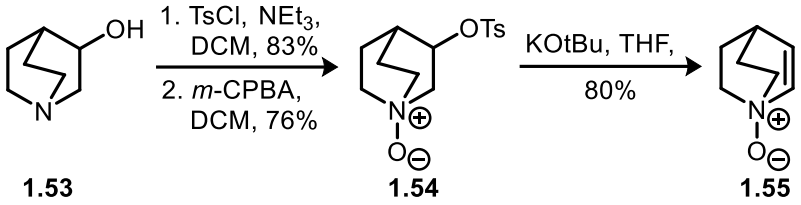
Since this first occurrence two routes into enamine *N*-oxides have been developed. The first is *via* the elimination of HX from *β*-substituted *N*-oxides, such as **1.51** shown in Scheme 1.13. First reported by Krouwer and Richmond in 1977 and further developed and utilised by Woodward and co-workers, shown in Scheme 1.13, and within the O'Neil group.[78, 79]



Scheme 1.13: Synthesis of enamine *N*-oxides *via* a HX elimination rection.

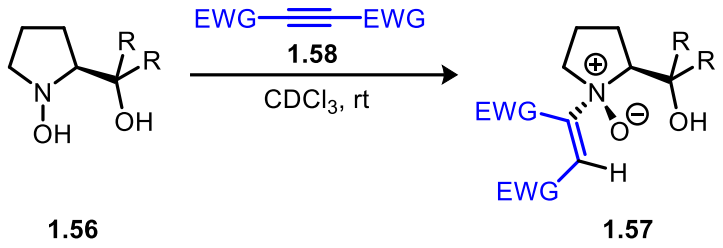
Synthesis was achieved by epoxide ring opening of **1.48** with an amine **1.49**, followed by chlorination and oxidation of the resulting amino alcohols **1.50** which gave the *β*-substituted *N*-oxide species **1.51**. Subsequent treatment with KO^{t}Bu prompted elimination to the desired enamine *N*-oxide **1.52**.

This same method has been applied to work within this group for the synthesis of quinuclidine enamine *N*-oxide **1.55** (Scheme 1.14). On this occasion, tosylation of the quinuclidine hydroxyl group, followed by oxidation of the tertiary amine to give the *β*-substituted quinuclidine *N*-oxide **1.54**. Treatment, once again with KO^{t}Bu, promoted elimination of tosic acid to affording quinuclidine enamine *N*-oxide **1.55**.[80]



Scheme 1.14: Synthesis of quinuclidine enamine *N*-oxide.

The second identified method of enamine *N*-oxide synthesis, developed within this group from Winterfeldt and Krohn's original observations in 1969, uses an intermolecular reverse-Cope cycloaddition reaction.[81] Reaction of chiral, prolinol derived hydroxylamines **1.56** with alkynes activated with electron withdrawing groups **1.58**, underwent a diastereoselective cycloadditon to give the chiral enamine *N*-oxides **1.57** (Scheme 1.15).



Scheme 1.15: Synthesis of chiral, proline derived enamine *N*-oxides *via* an intermolecular reverse-Cope elimination.

Enamine *N*-oxides have also been identified as intermediates, made by an intramolecular version of this reaction, in the synthesis of nitrones.[82] However, to date the limited number of publications discussed in this section are the only known syntheses of stable enamine *N*-oxide compounds.

1.2. *β*-Turn Mimetics and Peptidomimetic Design

Work in this thesis is aimed at the synthesis of a *β*-turn mimetic template. This section aims to give a brief background to the theory, development and application of *β*-turn mimetics.

1.2.1. Amino Acids and Protein Structure

"The structure, function and metabolism of all cells and tissues rely on the presence of specific proteins", which makes proteins and their precise structure essential to sustaining life.[83] Each protein possesses its own unique sequence of amino acids; the monomers of which make the polypeptide chain. This distinctive amino acid order is what leads to a protein's native structure, which ultimately allows the protein to carry out its physiological role within the body.

Amino acids have the chemical structure H_2N-CRH-CO_2H, where R is the side chain, situated on the *α*-carbon, which gives each amino acid its particular properties. All amino acids, with the exception of glycine where R=H, are chiral. There are 21 genetically encoded amino acids in humans, which with the exception of cysteine and selenocysteine are all of found in their *S*-form.

A proteins structure can be broken down into three distinct categories: primary, secondary and tertiary. Each stage of folding is influenced by the concluding structure of the previous stages and the final structural form of the protein is determined by the amino acid sequence, as well as a number of physiological conditions such as pH, solvent, presence of metal ions and prosthetic groups (groups other than amino acids) amongst others.[84]

The primary structure of any protein is the straight chain amino acid sequence encoded by the gene responsible for the proteins synthesis.[85] The secondary structure of a protein is a regular, repeating folding of the primary polypeptide chain. These folding interactions are held together by H-bonds primarily between the amide carbonyl of one amino acid residue and the amide NH of another. The two most common secondary structures are the *α*-helices and the *β*-pleated sheets.

An *α*-helix is a structure in which the polypeptide chain coils in a counter clockwise manner, with 3.6 amino acid residues in each turn of the helix and the amino acid side chain residues held pointing outwards. Each turn is about 5.4 Å long and is stabilised

by a hydrogen bond between one amino acid and another 4 positions further along the chain.[86]

The β-pleated sheet is made up of two or more polypeptide chains which line up next to each other, held together by H-bonds between amide bonds of the different chains. The polypeptide chains can either be running in the same direction, in parallel β-sheets, or in the opposite direction to one and other, in anti-parallel β-sheets (Figure 1.8).[86]

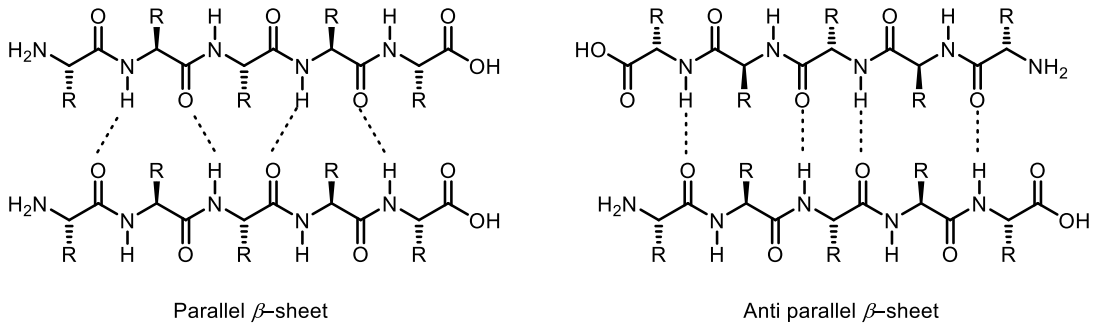


Figure 1.8: Structure of the two types of β-sheets.

Certain amino acid residues have a propensity to favour or disfavour structures due to the properties of their side chains. For example, the ring structure of proline, leading to its unusual dihedral angle for an amino acid, makes it highly disfavoured for it to be incorporated into either α-helices or β-pleated sheets. For this reason proline is often referred to as an α-helix breaker.[87]

Tertiary structure folding is primarily driven by the R groups of the present amino acids forming interactions with each other. These interactions include H-bonding, VDW-interactions, salt bridges between fully charged groups, and sulfide bonds between two cysteine residues.[85] One other important factor when a protein folds into its tertiary structure is hydrophobic interactions.[88] Hydrophobic residues cluster together inside the protein leaving hydrophilic regions of the structure on the outer edges to interact with the surrounding water molecules.

If a protein is made up of more than one polypeptide then the multiple tertiary structures can come together in one last energy minimising process to form what is called the quaternary structure.

1.2.2. *β*-Turns

β-Turns are the most common non-repetitive motif observed in the secondary structures of proteins, and they reverse the direction of the polypeptide chain.[89] They consist of 4 amino acid residues, with a stabilising hydrogen bond between the first *i*, and third, *i*+3 residues holding the C-*α* carbons of these residues at a distance of 7Å or less (Figure 1.9).[90] *α*-Turns and *γ*-turns are also possible, differing in their hydrogen bonding interactions and number of amino acid residues. *β*-Turns can be further broken down into different types, categorised by both their *Φ*-angles and *Ψ*-angles.[91]

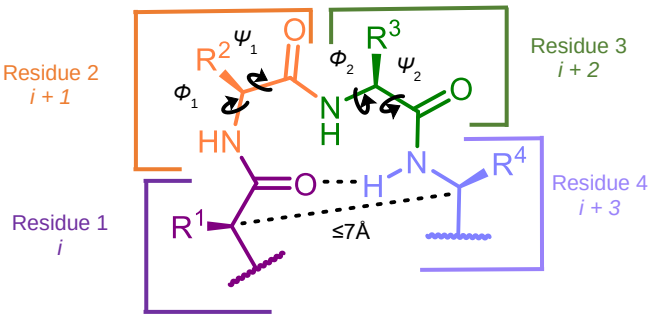


Figure 1.9: Generic *β*-turn structure.

If a *β*-turn is held between two *β*-strands it is termed a *β*-hairpin, the basic building block for *β*-sheets.[92] However the term *β*-hairpin can also refer to *α*-turns and *γ*-turns in the same position so they should be interchanged with caution. Because turns occur frequently between regions of secondary structure they are often located on the surfaces of proteins. This exposes the residues, which protrude out from the turn, to the aqueous environment. Consequently, *β*-turn amino acid residues are predominantly hydrophilic with glycine, asparagine and aspartic acid among the most commonly found residues in turns.[88, 93] Proline, despite its non-polar nature, is also often found in the *i*+1 or *i*+2 positions of *β*-turns due to the presence of a ring structure.[93] Because of their surface exposed location *β*-turns are more likely to be part of molecular recognition sites for physiological processes such as ligand binding, protein-protein and protein-nucleic acid interactions.[89, 94]

1.2.3. *β*-Turn Mimetics by Peptidomimetic Design

Because *β*-turns occur frequently in the molecular recognition sites of those proteins, which are vital in every physiological process in the body, mimicking *β*-turns synthetically as a method of therapeutic design has become a subject of much research in modern chemistry.[94] *β*-Turns as units, are amenable to mimetic design due to their relatively small size, especially in comparison to *α*-helices and *β*-sheets. A *β*-turn mimetic is a molecule which shares the pharmacophoric features of the native turn held in the correct conformation, but with an altered non-peptide backbone framework.

The purpose of these peptidomimetic structures is to maintain, or enhance, the natural activity of the native *β*-turn whilst removing as many of the pitfalls of peptides being used as therapeutics as possible. These pitfalls include: poor subtype selectivity, poor biostability, rapid clearance and unfavourable absorption properties.[95]

Peptidomimetic design of *β*-turns from the native peptide can be approached from numerous directions. These approaches can be broken down into three main classes: Amino acid modifications, introduction of global restrictions to the natural protein and synthetic backbone scaffolds.[83] A combination of these methods can be applied, or alternatively they can be used in isolation of each other to reach the mimetic structures.

The focus of mimetic design in this thesis aims to develop a synthetic backbone consisting of a bicyclic morpholine template to mimic a *β*-turn. This method has been used frequently in the past in the design and synthesis of *β*-turn mimetics. The aim of this method is to create a cyclic or bicyclic template based on the native turn which is being mimicked, which introduces increased stability and rigidity by removing the peptide backbone. These cyclic templates can be furnished with *C* and *N*-termini which are by design orientated in the same torsional angles of the natural peptide chain. These can be extended where required to increase peptide-like character and increase compound affinity to its target. The templates can also be further functionalised at alternate points on the systems in attempts to increase target site affinity. A select few examples of cyclic and bicyclic *β*-turns are shown in Figure 1.10 which use the methods discussed to elicit their response.[96-100]

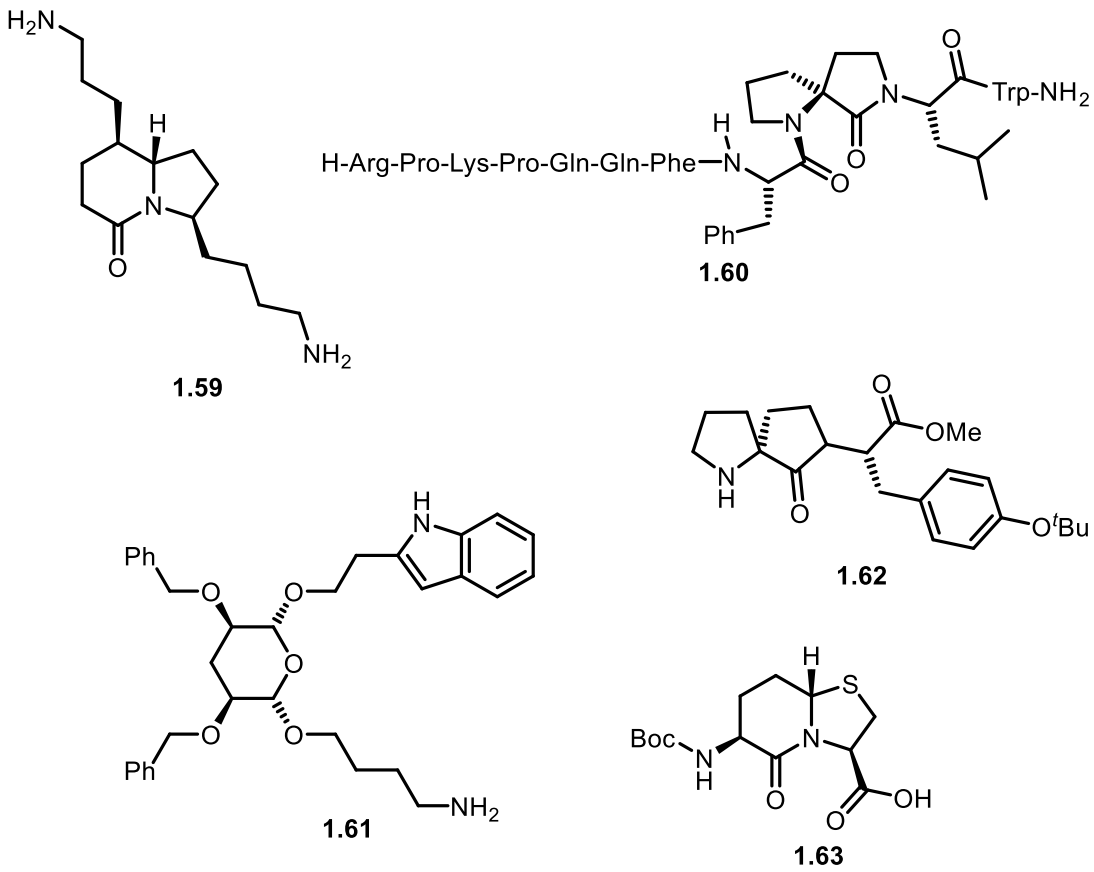


Figure 1.10: *β*-Turn mimetic structures **1.59**, **1.60**, **1.61**, **1.62** and **1.63**.

Chapter 2

The Synthesis of Fused Morpholine Enamine *N*-Oxides as Potential *β*-Turn Mimetics

2. Chapter 2 - The Synthesis of Fused Morpholine Enamine *N*-Oxides as Potential *β*-Turn Mimetics

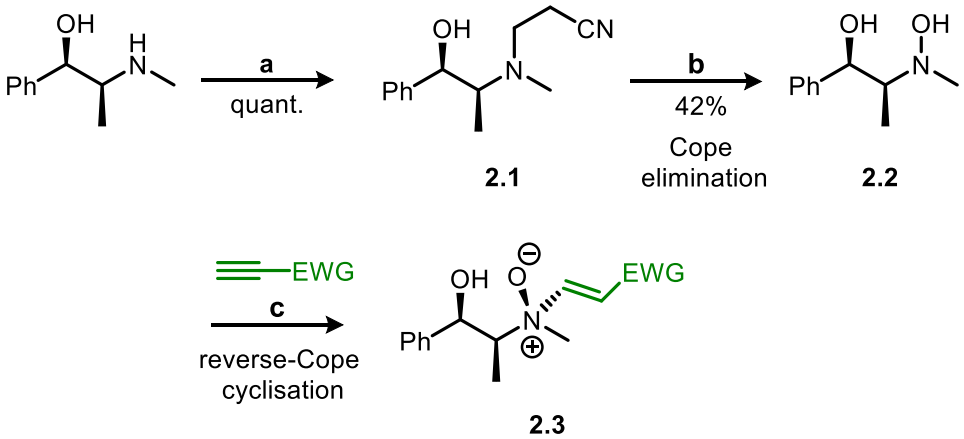
2.1. Introduction to Project

This chapter describes the synthesis of a variety of novel fused morpholine enamine *N*-oxides *via* the tandem Cope elimination/reverse Cope cyclisation reaction. The application of these fused morpholine systems in an active industrial protein-protein interaction (PPI) project will also be outlined and discussed.

2.1.1. Chiral Enamine *N*-Oxides *via* an Intermolecular Reverse-Cope elimination

Enamine *N*-oxides, which are described in more detail in section 1.2., are an especially rare functional group, and there are only a handful of publications in the literature describing their synthesis and properties. Of the limited reported literature on this subject a significant amount has been performed within the O'Neil research group. One such example of this is depicted in scheme 2.1, were a series of ephedrine derived, chiral enamine *N*-oxides were synthesised *via* an intermolecular reverse-Cope cyclisation.

Ephedrine was treated with acrylonitrile in methanol to yield the *N*-cyanoethyl adduct **2.1** in quantitative yield. *N*-oxidation with *m*-CPBA triggered Cope elimination to yield the hydroxylamine **2.2** in a 42% yield, which upon treatment with a series of alkynes underwent an intermolecular reverse-Cope elimination to give the chiral enamine *N*-oxides **2.3** in a >20:1 mix of diastereoisomers.[81]



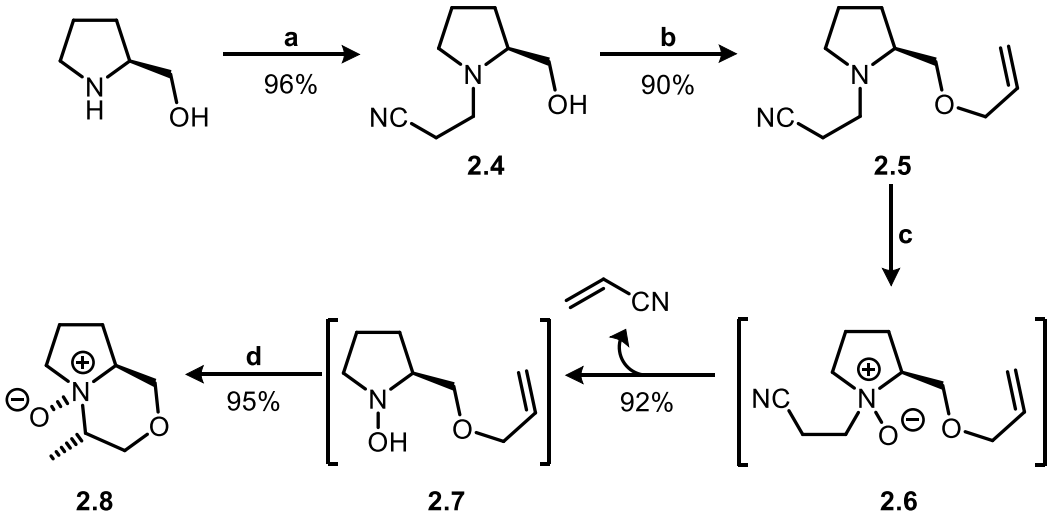
Scheme 2.1: Ephedrine derived enamine *N*-oxide synthesis by intermolecular reverse-Cope elimination. *Reagents and Conditions* **a-** acrylonitrile, MeOH **b-** *m*-CPBA, K_2CO_3, DCM **c-** $CDCl_3$.

The lack of knowledge surrounding this functional group and the further development of this synthetic methodology is one of the primary motivations for the work covered in this chapter.

2.1.2. The Tandem Cope Elimination/Reverse Cope Cyclisation Reaction

As was discussed in section 1.1.2.2., the reverse-Cope cyclisation reaction is one of the more widely used methods for the synthesis of tertiary amine *N*-oxides. Within the O'Neil research group this reaction, in tandem with the Cope elimination reaction, has been utilised extensively. Most relevant to the work in this thesis is the use of this tandem reaction for the synthesis of chiral, functionalised morpholine *N*-oxides.[101] This protocol has also been used for the synthesis of bicyclic lactam and lactone *N*-oxides.[101, 102]

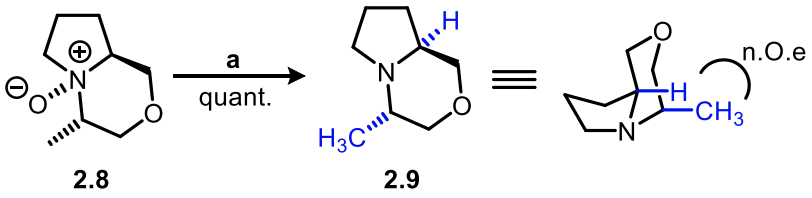
Motivated by earlier research on the reverse-Cope cyclisation reaction, the tandem Cope elimination/reverse-Cope cyclisation reaction was used to synthesise the *N*-oxide **2.8** (Scheme 2.2).[101, 103] *(S)*-Prolinol underwent conjugate addition with acrylonitrile to yield the cyanoethyl furnished derivative **2.4**. *O*-Allylation with allyl bromide then gave the tertiary amine **2.5**, with a suitably positioned *β*-hydrogen and alkene require for the tandem reaction.



Scheme 2.2: Synthesis of fused morpholine *N*-oxide. *Reagents and Conditions* **a-** acylonitrile (1.1 eq.), MeOH, 0 °C, o/n. **b-** (i) NaH (1.1 eq.), THF, 0 °C, 30 mins (ii) allyl bromide (1.1 eq.), o/n **c-** *m*-CPBA (1.1 eq.), K_2CO_3 (1.5 eq.), DCM, -78 °C, o/n **d-** MeOH, reflux, 2 days.

Oxidation of the tertiary amine **2.5** with *m*-CPBA produced the *N*-oxide intermediate **2.6**, which underwent Cope elimination *in situ* to give the hydroxylamine **2.7**. Heating in MeOH induced the reverse-Cope cyclisation of the hydroxylamine **2.7** to afford the fused morpholine *N*-oxide **2.8** in 76% yield over the 4 steps.

The cyclisation occurred stereoselectively to give a single diastereomeric product and introducing two new stereocenters in one step. The exact structure of the reverse-Cope elimination product was established by nOe experiments on the corresponding morpholine **2.9**, synthesised by the hydrogenation of the fused morpholine *N*-oxide in the presence of a palladium catalyst (Scheme 2.3). An nOe was observed between the C-α proton and methyl CH_3, shown in blue, confirming the methyl group was situated on the bottom face of the system. Therefore, due to the concerted nature of the reverse-Cope cyclisation, the methyl group and *N*-oxide oxygen must be *syn* to each other in the product, hence the *N*-oxide **2.8** was assigned the structure shown in scheme 2.3.

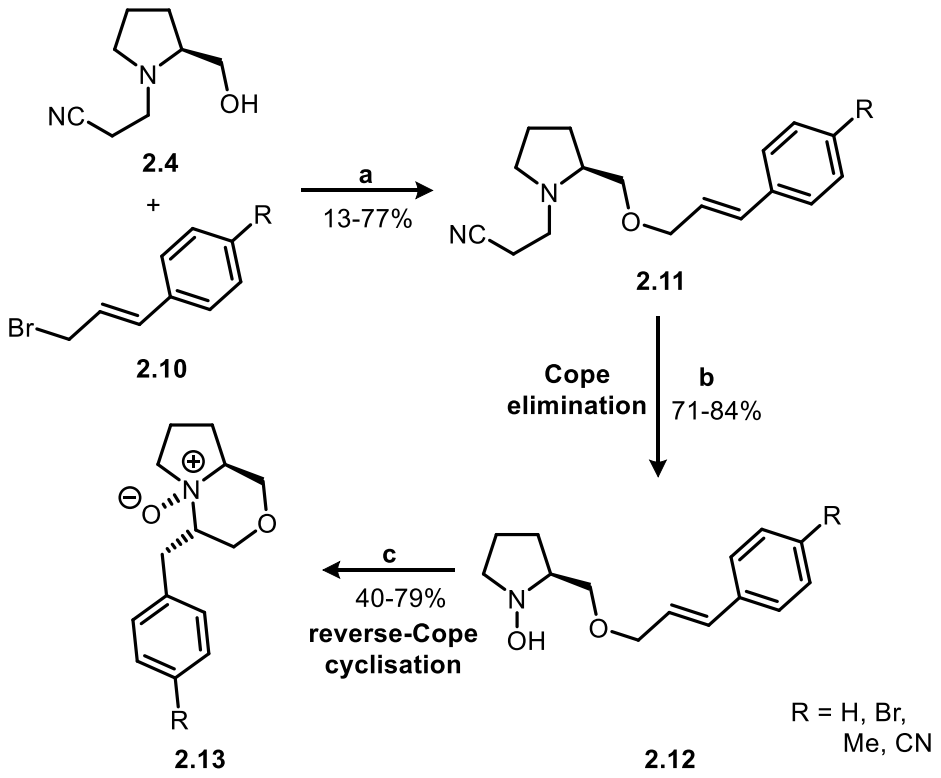


2.8 **2.9**

Scheme 2.3: Hydrogenation of fused morpholine *N*-oxide. *Reagents and Conditions* **a-** H_2, Pd/C (cat.), MeOH.

2.1.3. Scope of Tandem Cope Elimination/Reverse Cope Cyclisation Reaction

Additional work was also carried out on this fused morpholine *N*-oxide to investigate the functionality that could be incorporated into the chiral product.[104] Allyl bromide was replaced with a series of cinnamyl bromide derivatives **2.10**, which upon allylation of *N*-cyanoethyl prolinol **2.4** gave the tertiary amine species **2.11** (Scheme 2.4). Subsequent Cope elimination/reverse-Cope cyclisation reactions gave the fused morpholine *N*-oxides **2.13**.



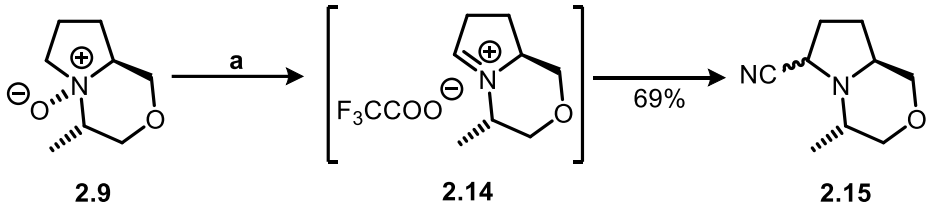
Scheme 2.4: Synthesis of functionalised fused morpholine *N*-oxides. *Reagents and Conditions* **a-** (i) NaH (1.1 eq.), THF, 0°C, 30 mins (ii) **2.10** (1.1 eq.), TBAI (cat.), o/n **c-** *m*-CPBA (1.1 eq.), K_2CO_3 (1.5 eq.), DCM, -78 °C, o/n **d-** MeOH, Δ, 2 days.

Any substituent on the terminus of the alkene showed significant slowing of the reverse-Cope cyclisation step, as well as giving lower overall yields. Also, only phenyl or *para*-substituted phenyl substituents were tolerated, compounds with terminal alkyl substituents failed to undergo cyclisation. These results were in accordance with the observations discussed by Knight and Cooper in their review on the reverse-Cope cyclisation, in which they state that "distal alkene substituents underwent cyclisation significantly slower than non-substituted derivatives".[36]

2.1.4. Pyrrolidine Ring Functionalisation by the Polonovski-Potier Reaction

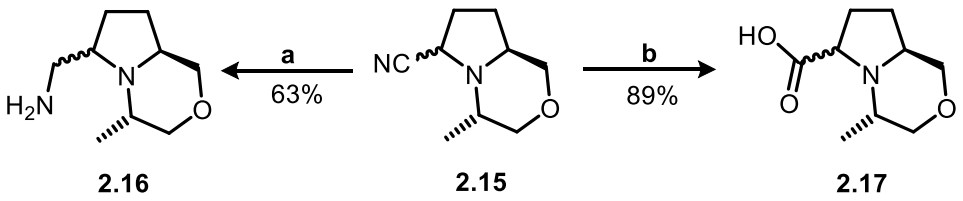
Functionalisation of the pyrrolidine ring in the fused system, by a modified Polonovski reaction, has also been investigated.[105] The modified Polonovski, or Polonovski-Potier reaction, was initially used to synthesise an α-amino nitrile **2.15** from the fused morpholine *N*-oxide species **2.9** (Scheme 2.5).

Treatment of the *N*-oxide **2.9** with TFAA gave the iminium ion intermediate **2.14**, which could be isolated and stored at low temperatures. This was then trapped out with aqueous KCN to give the α-amino nitrile **2.15** in a 69% yield, as a mixture of diastereoisomers. These could be separated and isolated by column chromatography, but would epimerise rapidly in solution by loss of the nitrile group and addition back into the planar iminium ion **2.14**.



Scheme 2.5: Polonovski-Potier reaction of fused morpholine *N*-oxide. *Reagents and Conditions* **a-** (i) TFAA (2.0 eq.), DCM, -15 °C, 90 mins (ii) KCN (1.5 eq.), 30 mins.

Reduction and hydrolysis of the α-amino nitrile species **2.15** gave the primary amine **2.16** and the carboxylic acid **2.17** respectively, shown in scheme 2.6. In both cases the products were obtained as a mixture of diastereoisomers due to the diastereoisomeric nature of the α-amino nitrile starting material.

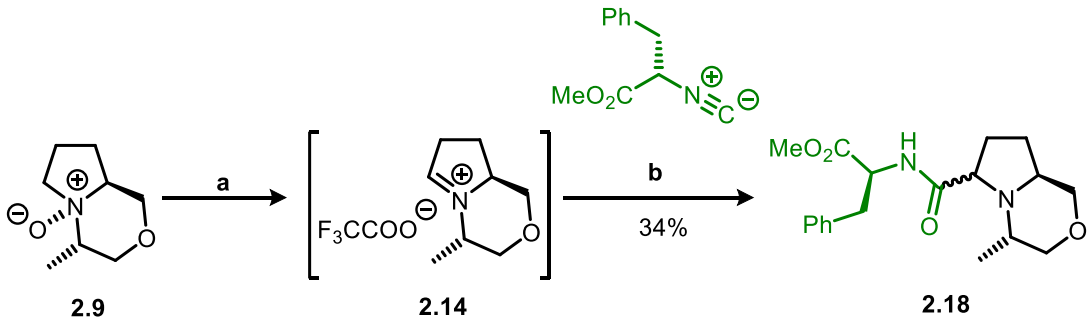


Scheme 2.6: Hydrolysis and reduction of nitrile terminus. *Reagents and Conditions* **a-** $LiAlH_4$ (2.0 eq.), ether, reflux, 4h **b-** conc. HCl, 50 °C, 8h.

Additionally, the iminium ion intermediate **2.14** could also be trapped out with Grignard reagents to furnish the pyrrolidine ring with alkyl or allyl groups, or organolithiums to give acetate or benzoyl substituents at the α-position.

A particularly interesting application of his system carried out in the O'Neil group, in regards to the work carried out in this chapter, was the trapping out of the imnium ion

intermediate with amino acid derived isocyanides (scheme 2.7).[106] Use of an isocyanide in this reaction is an Ugi variation of the Polonovski-Potier reaction.[107, 108] *N*-Oxide **2.9** was treated with TFAA in DCM to generate the iminium ion *in situ*, as previously described. The iminium ion intermediate **2.14** was trapped out with phenyl alanine methyl ester isocyanide, which was synthesised from the natural *L*-amino acid, to give the α-functionalised tertiary amine **2.18** in a 34% yield as a mixture of diastereoisomers after basic work-up.[109]



Scheme 2.7: Polonovski-Potier reaction of fused morpholine *N*-oxide with amino acid derived isocyanides. *Reagents and Conditions* **a-** TFAA (2.0 eq.), DCM, -15 °C, 90 mins **b-** (i) DCM, 30 mins (ii) NaOMe (1.5 eq.), MeOH.

The peptide like structure **2.18** was also synthesised by an unambiguous route, in which peptide coupling of the previously synthesised carboxylic acid **2.17** with phenyl alanine methyl ester provided the identical species **2.18**, proving that isocyanide addition had occurred at the *α*-position, as suspected.

2.2. Fused Morpholine Bicyclic System as a *β*-Turn Mimetic

As mentioned in section 1.2. mimicking *β*-turns found in natural protein structures has been an area of increasing research in the field of peptidomimetics. One of the principle methods of *β*-turn peptidomimetic design is global restriction of turns by synthetic backbone design. This chapter aims to discuss the application of the bicyclic morpholine template, *vida infra*, as the basis of a *β*-turn peptidomimetic, which has been applied to an active PPI project with our industry sponsors LifeArc. Full project details of which are covered in section 2.2.2..

2.2.1. Modelling of Fused Morpholine System as Potential *β*-Turn Mimetic

Some initial molecular modelling work was carried out by the Berry group at the University of Liverpool, integrating the bicyclic morpholine structure into a potential peptidomimetic.[110] Amine and carboxylic acid termini were added to the bicyclic morpholine template at the previously functionalised positions, which were then overlaid on a generic *β*-turn as shown in figure 2.1.

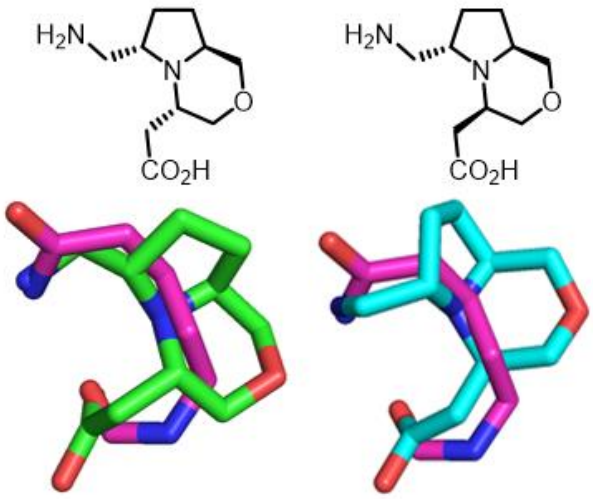


Figure 2.1: Proposed *β*-turn mimetic template (in green and turquoise) overlaid on a generic *β*-turn structure (in pink) showing high degree of overlap.

The molecular modelling conducted suggested a good overlay of the bicyclic morpholine template and the *β*-turn structures. Specifically, the amine and acid termini of both morpholine structures were held in similar torsional angles compared to the generic turn. The cyclic system of the template holds the two terminal groups rigidly

in these orientations, providing potential as a synthetic β-turn peptidomimetic, which could be built upon for specific utility.

2.2.2. LifeArc PPI Project Outline

Working in collaboration with our industrial sponsors LifeArc, the bicyclic morpholine template was applied to an active in house project. The project aim was to target a protein-protein interaction (PPI), between a cell surface protein and its activating ligand. It was hoped to identify a small molecule, β-turn mimetic inhibitor of the PPI being targeted.

2.2.3. PPI Target Site

Due to a confidentiality agreement with our industrial sponsors the identity and some specific details of the work carried out in this chapter have been withheld.

The interaction of the two proteins of interest has been the subject of much research. This work has shown that the activating ligand binds to the cell membrane protein as a dimeric species, of which a crystal structure of the binding interaction between the two proteins is shown in figure 2.2. Binding occurs between a homodimer of the ligand and a concave interface formed by domains 1 and 2 of the cell surface protein.

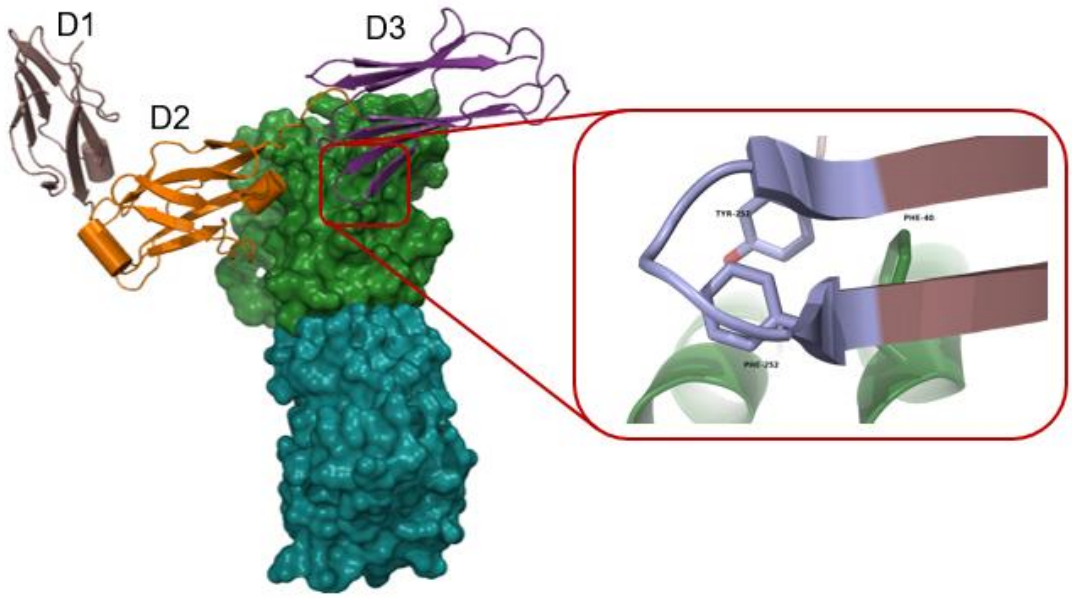


Figure 2.2: Target PPI crystal structure. Activating ligand dimer is shown in green and turquoise. Cell surface membrane protein domains 1-3 shown in brown, orange and purple. Key interaction site zoomed in (right), showing key β-turn of cell surface protein and Phe-40 residue of activating ligand.

A key region of the binding interface was identified as a hairpin turn of D3 of the cell surface protein packing against the surface of the activating ligand. A hydrophobic area is formed within this interaction between Phe-40 of the ligand protein, and Tyr-257 and Phe-252 of the cell surface protein. Alanine scanning, a technique which systematically replaces amino acids with an alanine residue, found that a significant lowering in binding was observed when Phe-40 was replaced with Ala-40.[111, 112] Phe-252 and Tyr-257 residues are situated at the ends of a 6 amino acid sequence, the central 4 of which create a *β*-turn. The full amino acid sequence of this portion of the surface protein was shown to be Phe-His-Asn-Asn-Arg-Tyr.

It was proposed that significant inhibition of the PPI could be achieved by interfering with the key site identified at the 3rd domain. Molecular modelling, carried out at LifeArc suggested that derivatives of the bicyclic morpholine template, discussed in section 2.1, could potentially be used as a mimetic of the *β*-turn which takes part in this key interaction.[113] By structurally imitating this turn sequence a mimic could potentially bind to the same binding site as the activating ligand, leading to a reduced binding interaction between the two proteins, resulting in reduced activation.

As a result of the modelling work that was carried out a number of series of compounds based on the fused morpholine template, shown in figure 2.3, were identified as targets. One series would possess the *N*-terminus chain (shown in blue) and a second would possess the *C*-terminus chain (shown in green). Finally, a third series with both *N* and *C*-termini substituents were identified. Each terminus could exist as 2 diastereoisomers, making a total of 4 when both termini are attached simultaneously.

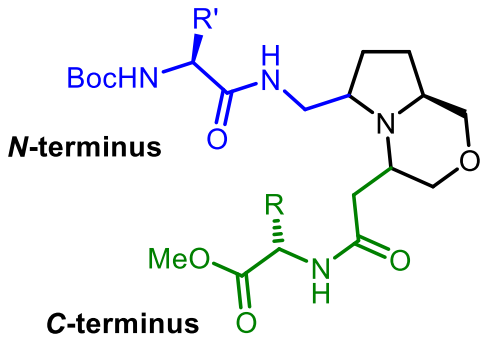


Figure 2.3: Basic β-turn mimetic template, with suggested template chain extensions shown in blue and green.

Synthesis of each series would aim to give the final compounds as single diastereoisomers initially, so that results in biophysical testing would give the maximum information about the binding interaction.

2.2.4. SPR Compound Screening

The bicyclic morpholine derived series was to be tested for their interaction with the activating ligand protein by a biophysical method called surface plasmon resonance (SPR). SPR is a phenomenon that occurs when polarised light is reflected off a conduction film positioned between two substances with a different refractive index. SPR biosensors have established themselves as an efficient mode of observing protein-protein interactions as well as protein-inhibitor interactions.[114-116] Depicted below is a generalised image of the SPR experiment (Figure 2.4), which was taken from a publication by Meyerkord and Fu on the testing of PPIs.[117]

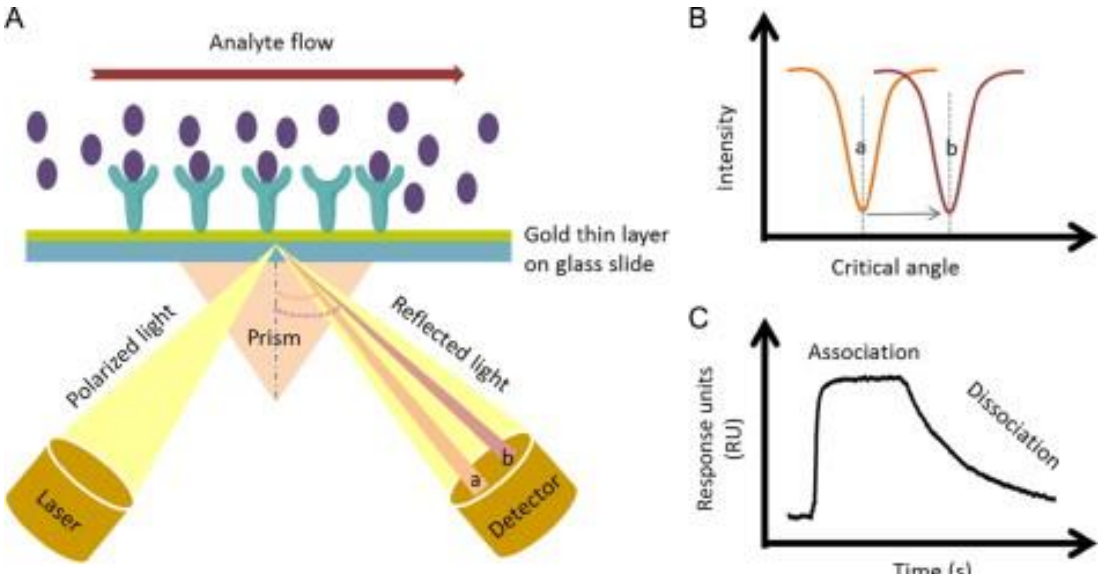


Figure 2.4: SPR method summary.

In SPR testing a protein, in this case the activating ligand, is immobilised onto a chip coated with gold. An analyte is then passed over at varying concentrations so that the interaction, if any can be monitored. The SPR instrument measures the change in refractive index (or critical angle), shown in graph B, of the solvent near the chip surface of the immobilised protein during the association dissociated process.[118] The instrument does this by shining polarised light through a prism onto the bottom of the

chip coated with protein. It is the change in the polarised light angle which gives the critical angle change when it hits the detector.

The observed change in refractive index is related to the change in mass on the surface of the chip, which is caused by the binding of the analyte to the immobilised protein. The signal is given in resonance units (RU) which is proportional to the change in mass.[119] Also taken into account when calculating the RU is the amount, and the activity of the immobilised protein. Once the protein is immobilised onto the gold chip it will have a maximum binding capacity (R_{max}). This can be calculated by the equation:[120]

$$R_{max} = MW\ analyte / MW\ ligand \times S_m \times R_L$$

From this equation it can be seen that R_{max} is dependent on the molecular weight of the immobilised ligand and the analyte, the amount of ligand on the chip, R_L, and the stoichiometry of the binding interaction between analyte and ligand, S_m. Experimental R_{max} is never as high as the theoretical R_{max} due to protein damage upon immobilisation or during regeneration between runs.

Testing the protein-β-turn mimetic interaction by the use of SPR has a number of advantages:[114]

- High sensitivity – even low level binding of small molecules can be detected
- Label-free detected – no analyte modifications are required that could affect binding, which also reduces synthetic demands
- Real-time monitoring of interaction – this can be used to determine the kinetics as well as the amount of binding
- A high degree of automation of the process can be used

With all of its advantages SPR does have one major disadvantage, the exact binding site of the analyte is not specified. Therefore, further tests must be carried out once the desired interaction has been established to fully understand the nature and site of binding. This can be done by X-ray diffraction of a protein:analyte co-crystal, or NMR experiments on crystals of the interaction of interest.

2.3. Project Aims

The aims of this chapter can be broken down into two main categories. Although there is some degree of overlap between the two areas.

Objective 1:

There is virtually nothing reported in the literature concerning the synthesis, stability and chemical reactivity of enamine *N*-oxides. The work in this chapter was aimed to advance previous work on the synthesis of enamine *N*-oxides and to develop novel methods for their synthesis. To do this the tandem Cope elimination/reverse-Cope cyclisation would be employed in an intramolecular fashion.

Objective 2:

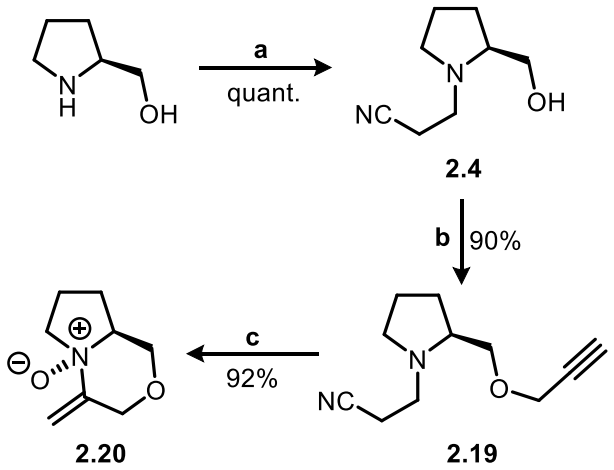
Routes into the functionalised bicyclic morpholine template were to be investigated. A method for the addition of substituents on the *N*-terminus on the pyrrolidine ring had already been established (Section 2.1.). For this reason the work in this chapter would focus mainly on the incorporation of the *C*-terminus acid handle onto the morpholine ring. Once a route to the acid functionalised template has been established, the synthesis of β-turn mimetic derivatives was planned and successful derivatives would be assessed by SPR biophysical testing. Additional points of diversity would also be introduced onto the template to increase future scope and possible uses for the ring system.

2.4. Results and Discussion

2.4.1. Alkyne Incorporation

The major hindrance to functionalisation of the morpholine ring using the reverse-Cope cyclisation was the limitation of the substituents that could be put onto the alkene, as discussed in section 2.1.3.. Incorporation of an alkyne, instead of the alkene, was proposed to circumvent this problem.

(S)-Prolinol was used as the starting material for the synthesis, as it had been used for the synthesis of the fused morpholine template, as well as introducing a chiral centre from the start of the synthesis.[101] Conjugate addition of acrylonitrile with *(S)*-prolinol in methanol afforded the *N*-cyanoethyl prolinol **2.4** in quantitative yield. The *N*-cyanoethyl prolinol **2.4** hydroxyl group was deprotonated with NaH and propargyl bromide added to give the propargyl ether **2.19** in a 90% yield. ^{1}H NMR showed the appearance of the distinctive alkyne terminal proton as a triplet at 2.43 ppm.



Scheme 2.8: Synthesis of morpholine enamine *N*-oxide. *Reagents and Conditions* **a-** acrylonitrile (1.1 eq.), MeOH, 0 °C **b-** (i) NaH (1.1 eq.), THF, 0°C, 30 mins (ii) propargyl bromide (1.1 eq.), o/n **c-** *m*-CPBA (1.1 eq.), K_2CO_3 (1.5 eq.), DCM, -78°C, o/n.

Treatment of the tertiary amine **2.19** with *m*-CPBA in DCM led to tandem Cope elimination/reverse-Cope cyclisation which afforded enamine *N*-oxide **2.20** as a single diastereoisomer in a 92% yield after column chromatography (Scheme 2.8). The enamine CH_2 appeared as two distinct singlets at 6.00 and 5.22 ppm. The enamine *N*-oxide was obtained as a hygroscopic semi solid, which was stored for prolonged

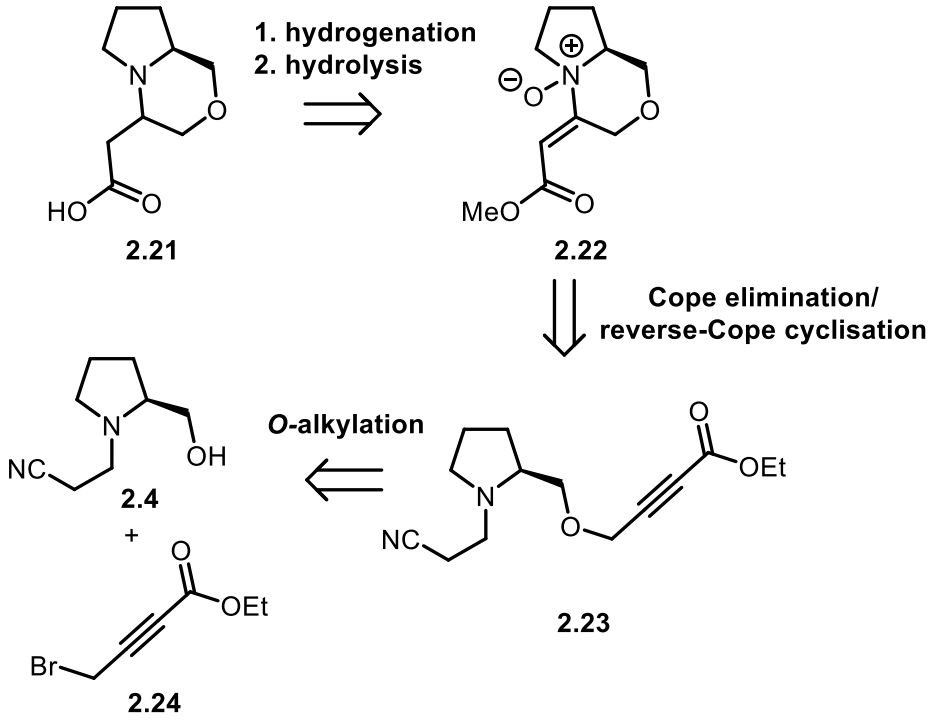
periods of time in a desiccator over P_2O_5. The tertiary amine *N*-oxide structure was assigned by analogy to compounds discussed in section 2.4.4..

There were several major differences between the alkene and alkyne analogues when they underwent the tandem reaction protocol. There was no observable hydroxylamine intermediate in the reverse-Cope cyclisation observed in the case of the alkyne **2.19**, unlike in the alkene analogue. The reaction of the alkyne proceeded at ambient temperature in DCM, compared to the alkene which required heating in MeOH.

To the best of our knowledge this is the first example of the tandem Cope elimination followed by an **intramolecular** reverse Cope cyclisation for the synthesis of enamine *N*-oxides. Similar procedures have been utilised in an intermolecular fashion, by Winterfeldt and Krohn, and also in this research group.[76, 81]

2.4.2. Alkyne Functionalisation

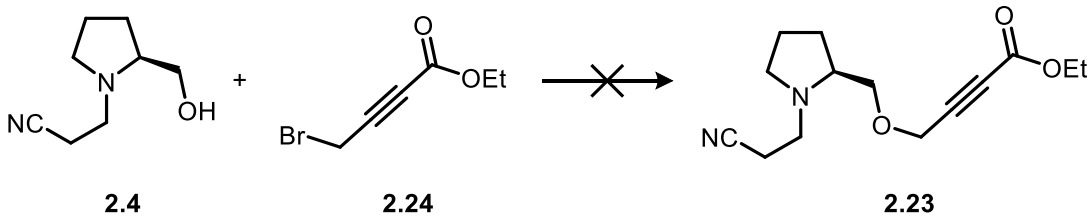
With the route to the parent enamine *N*-oxide established we then examined the synthesis of functionalised analogues. As discussed in section 2.2.3. the key intermediate in the design of these peptidomimetics possesses the *C*-terminus at the enamine terminal position. Therefore, using the same approach we had used with the alkene analogues (Section 2.1.3.), the retrosynthetic strategy shown in scheme 2.9 was proposed.



Scheme 2.9: Proposed retrosynthetic route to acid functionalised template.

The acid terminus could be obtained *via* hydrogenation and hydrolysis of *β*-enamino ester *N*-oxide **2.22** to give morpholine **2.21**. The synthesis of **2.23** using bromotetrolates in place of propargyl bromide should give the requisite precursors for cyclisation. It was decided the acid terminus would be protected until after the bicyclic system had been formed. It was thought a carboxylic acid being present in the system throughout could interfere with intermediate steps as well as make purification methods more challenging due to increased polarity.

Bromotetrolate **2.24** was prepared according to literature procedure from THP protected propargyl alcohol.[121] However this species proved to be highly unstable under the reaction conditions for *O*-alkylation of *N*-cyanoethyl prolinol **2.6** (Scheme 2.10). Upon addition of the bromide **2.24** to the alkoxide containing reaction mixture, the solution mixture immediately turned a dark brown colour. TLC showed the presence of numerous decomposition products, none of which could be indentified by crude NMR.



2.4 **2.24** **2.23**

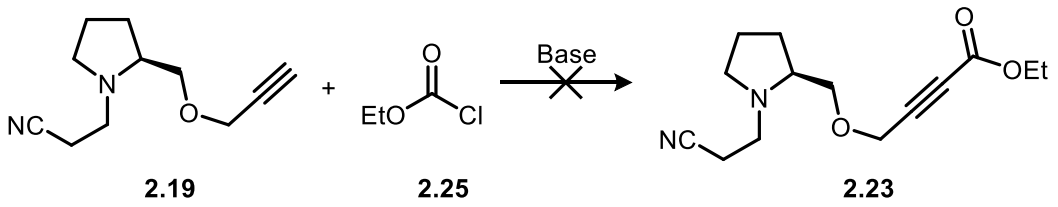
Scheme 2.10: *O*-propargylation of *N*-cyanoethyl prolinol. *Reagents and Conditions* **a-** (i) NaH (1.1 eq.), THF, 0 °C, 30 mins (ii) bromotetrolate (1.1 eq.),

2.4.3. Alkyne Functionalisation Post *O*-Alkylation

Due to the problems encountered attempting to alkylate the *N*-cyanoethyl prolinol **2.6** with pre-functionalised acetylenes, an alternative strategy was envisaged. Functionalisation of the tertiary amine **2.19** should give the propargyl ether, which could give access to the required compounds.

Once again, a protected acid was required on the alkyne terminus for the key *β*-turn intermediate. Tertiary amine **2.19** was synthesised as described previously. Attempts

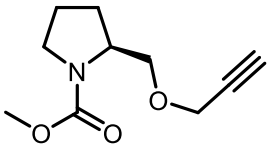
to form the propargylic ester **2.23** from the alkyne **2.19** by deprotonation of the alkyne and trapping the anion with chloroformate **2.25** were all unsuccessful (Scheme 2.11).



2.19 **2.25** **2.23**

Scheme 2.11: Attempted ester functionalisation of terminal alkyne.

Instead of the desired propargylic ester product **2.23**, a significantly less polar species was isolated. It was clear from the ^{1}H NMR of this unknown species that the terminal alkyne proton at 2.48 ppm was still present, but the cyanoethyl signals had disappeared. Carbamate **2.26**, shown in Figure 2.5, was identified as the compound obtained by 2D NMR analysis and mass spectrometry.



2.26

Figure 2.5: Unexpected compound identified as product from attempted propargylic ester **2.26** synthesis.

It is likely that the cyanoethyl group was lost *via* β-elimination to give the amine. Nucleophilic attack of the amine into the chloroformate then gave the observed carbamate species observed.

Due to the unsuccessful attempts to insert the acid handle by propargylic ester formation, pre or post *O*-alkylation of *N*-cyanoethyl prolinol, alternative routes were sought. Ester incorporation was still the ideal for later conversion to the corresponding acid in terms of β-turn mimetic synthesis. However, alternative functionality was explored as increasing the enamine *N*-oxide functionalisation was also a significant part of the research goal.

2.4.4. Triton B Reactions

Triton B (Figure 2.6), a quaternary ammonium hydroxide base, was identified as an alternative base to avoid the competitive *β*-elimination of acrylonitrile taking place. The benzyltrimethyl ammonium hydroxide has been shown to catalyse the alkynylation of ketones and aldehydes by Ishikawa and co-workers.[122] They had demonstrated that propargylic, terminal alkynes could undergo these reactions with a diverse range of carbonyls. It was proposed that the reaction was going *via* the ammonium acetylide as a reactive intermediate of this reaction.

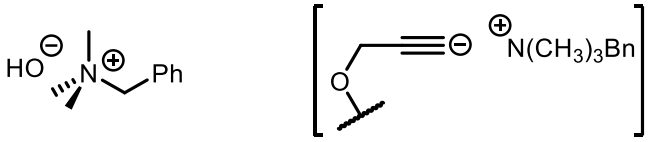
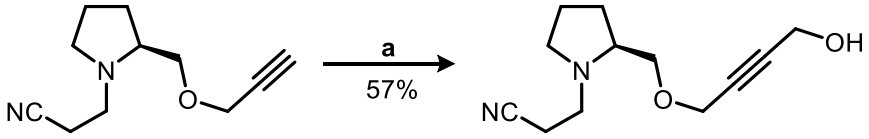


Figure 2.6: Triton B structure (left) and proposed ammonium acetylide intermediate (right).

The procedure from the aforementioned publication was applied to our alkyne species **2.19**. Alkyne **2.19** was treated with 10 mol. % Triton B in the presence of paraformaldehyde in DMSO to give **2.27** in 57% yield (scheme 2.12). The distinctive alkyne triplet at 2.43 ppm had clearly disappeared and an additional methylene peak had appeared at 4.30 ppm. It was clear that the reaction was not going to completion. Unreacted starting material was recovered from the reaction and recycled. Catalytic loading of Triton B and equivalents of paraformaldehyde were raised in an attempt to push the reaction to completion, however the initial reaction conditions were found to be optimal.

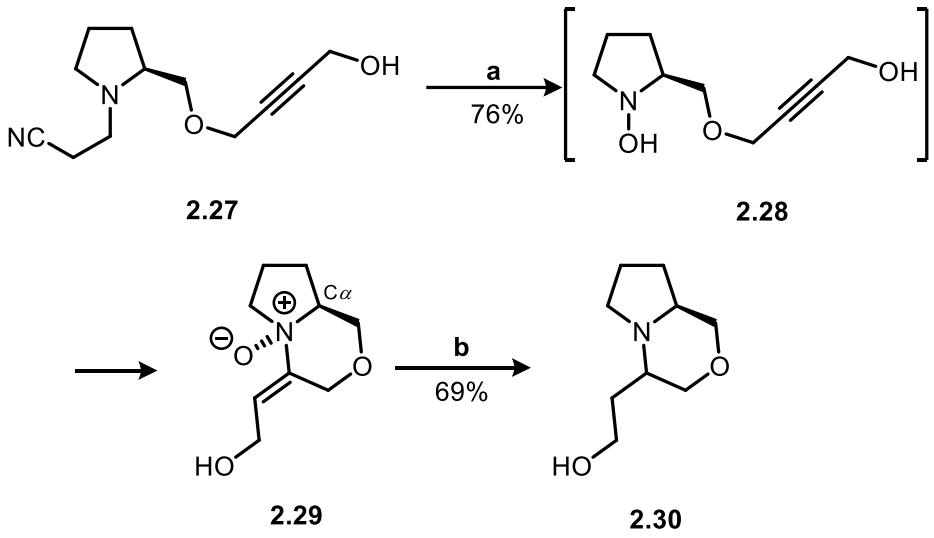


2.19 **2.27**

Scheme 2.12: Hydroxymethylene alkyne functionalisation. *Reagents and conditions* **a-** Triton B (0.1 eq.), paraformaldehyde (1.2 eq.), DMSO, rt, o/n.

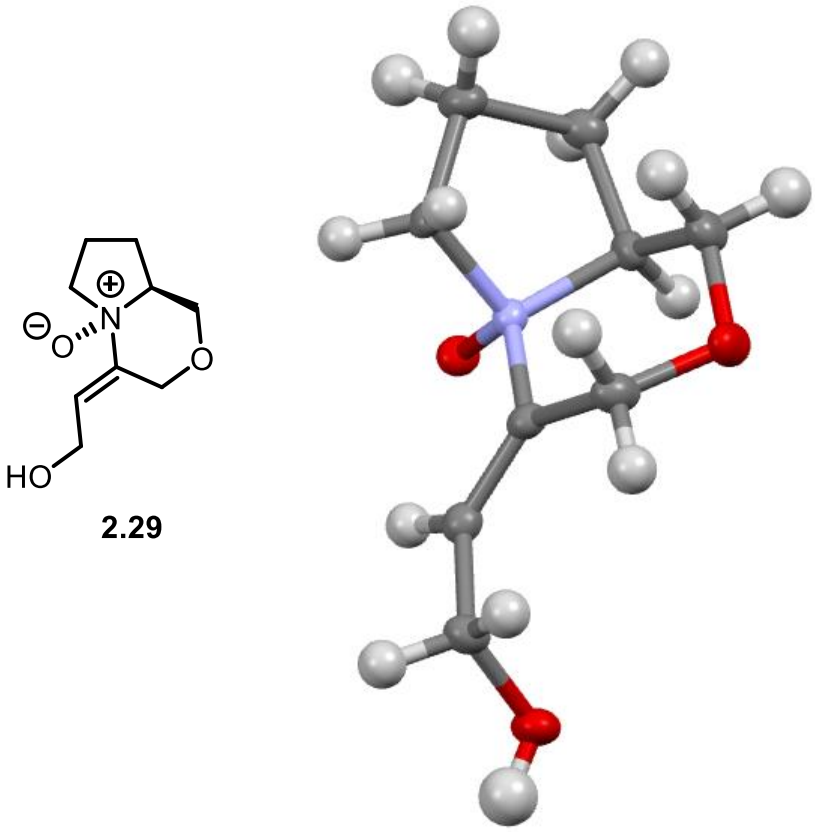
Oxidation of tertiary amine **2.27** with *m*-CPBA led to Cope elimination, and then reverse Cope cyclisation to give enamine *N*-oxide **2.29** in a 76% yield (Scheme 2.13).

A characteristic vinylic triplet was observed at 6.93 ppm as well as the distinguishing downfield shift of the C-*α* proton was also observed, from 2.75 ppm to 3.73 ppm.



Scheme 2.13: Enamine *N*-oxide formation and hydrogenation. *Reagents and Conditions* **a-** *m*-CPBA (1.1 eq.), K_2CO_3 (1.5 eq.), DCM, -78 °C, o/n **b-** H_2, Pd/C (0.1 eq.), MeOH, rt.

Enamine *N*-oxide **2.29** was formed as a single diastereoisomer as yellow crystals. An X-ray crystal structure was obtained of **2.29**, which is shown in figure 2.7. This showed that the *N*-oxide had formed exclusively on the bottom face of the bicyclic structure. By analogy with the ^{1}H NMR spectra it was also determined that the parent enamine *N*-oxide **2.2** had the same *N*-oxide configuration (see section 2.4.1.). The C-*α* protons of both species were identical, which could only be the case if the *N*-oxide oxygen was situated on the same face. It is not surprising that the diastereoselectivity of the reverse-Cope cyclisation leads to the enamine *N*-oxide with the oxygen on the lower face of the system, as the attack of the hydroxylamine is likely to come from below the alkyne, situated in the pyrrolidine side chain on the top face. Additionally, the geometry of the enamine double bond is fixed as the *trans*-isomer due to the concerted *syn* mechanism of the reverse-Cope cyclisation.

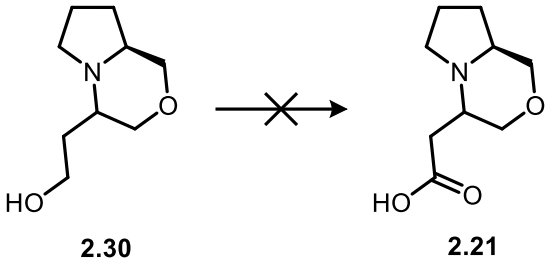


2.29

Figure 2.7: Crystal structure of methylene hydroxyl enamine *N*-oxide **2.29.**

Reduction of enamine *N*-oxide **2.29** was then carried out by hydrogenation in the presence of Pd/C in MeOH, which gave the tertiary amine **2.30** in a 69% yield. Interestingly, hydrogenation occurred with complete diastereoselectively, confirmed by ^{13}C NMR, however the selectivity of the hydrogenation could not be assigned

Having successfully incorporated a hydroxyl functionality onto the morpholine ring oxidation to the corresponding carboxylic acid was attempted. Various oxidation methods were investigated to oxidise alcohol **2.30** to carboxylic acid **2.21**, which used reagents including: TEMPO/bleach and $RuCl_3/NaIO_4$ combinations as well as Jones reagent (Scheme 2.14). These oxidation reactions were all followed by LC-MS, in which no acid peak was observed under any of the conditions. It was thought that the polarity of **2.21** and the basicity of the tertiary amine within the structure may be hindering the reactions taking place.



2.30 **2.21**

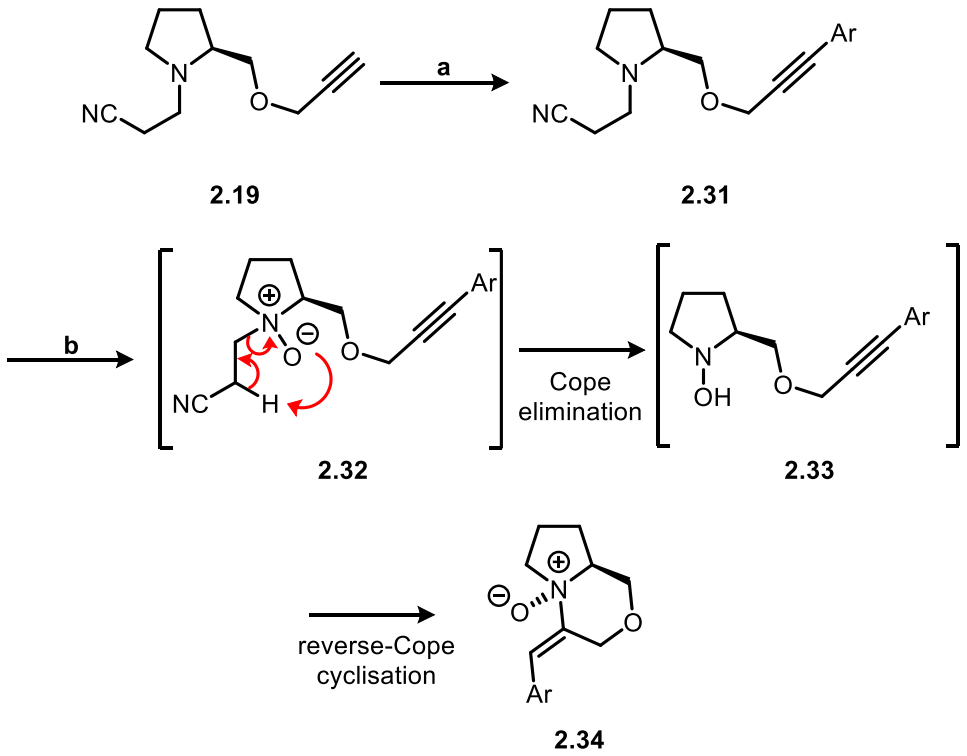
Scheme 2.14: Oxidation of hydroxyl chain.

This compound was unfortunately not carried forward towards any of the *β*-turn mimetic compounds for biological testing. However, this is the first example of the bicyclic morpholine template with a synthetically useful handle situated on the morpholine ring. Projects involving the further extension of this hydroxyl chain have been undertaken by other students within the group.

2.4.5. Sonogashira Cross Couplings

In order to attach different substituents onto the alkyne before reverse-Cope cyclisation we explored other methods of functionalisation. A Sonogashira cross coupling was also employed to functionalise the terminal alkyne of tertiary amine **2.19** (Scheme 2.15).

The Sonogashira cross coupling of terminal alkynes with vinyl or aryl halides, which utilises a palladium (0) catalyst and copper (I) co-catalyst, was first reported in 1975.[123] For the cross coupling of alkyne **2.19** with a number of vinyl iodides to give aryl functionalised terminal alkyne **2.31** $Pd(PPh_3)_2Cl_2$ and CuI were employed as the co-catalysts along with triethylamine as both solvent and base.



Scheme 2.15: Sonogashira cross coupling reaction and enamine *N*-oxide formation. *Reagents and Conditions* **a-** ArI (1.0 eq.), CuI (0.04 eq.), $Pd(PPh_3)_2Cl_2$ (0.01 eq.), NEt_3, rt, o/n **b-** *m*-CPBA (1.1 eq.), K_2CO_3 (1.5 eq.), DCM, -78°C, o/n.

The Sonogashira cross coupling proceeded *via* the catalytic cycle depicted in figure 2.8. The active palladium (0) catalyst is made by treatment of the $Pd(PPh_3)_2Cl_2$ pre-catalyst with triethylamine, which then undergoes oxidative addition with the aryl iodide to give **2.37**. Transmetallation between **2.37** and the copper co-ordinated terminal alkyne gave the palladium species **2.36**, which undergoes *trans-cis* isomerisation which is followed by reductive elimination to regenerate the active palladium catalyst and the aryl alkyne **2.31**. Good to high yields were obtained in all cross coupling reaction examples, and yields are summarised in Table 2.1.

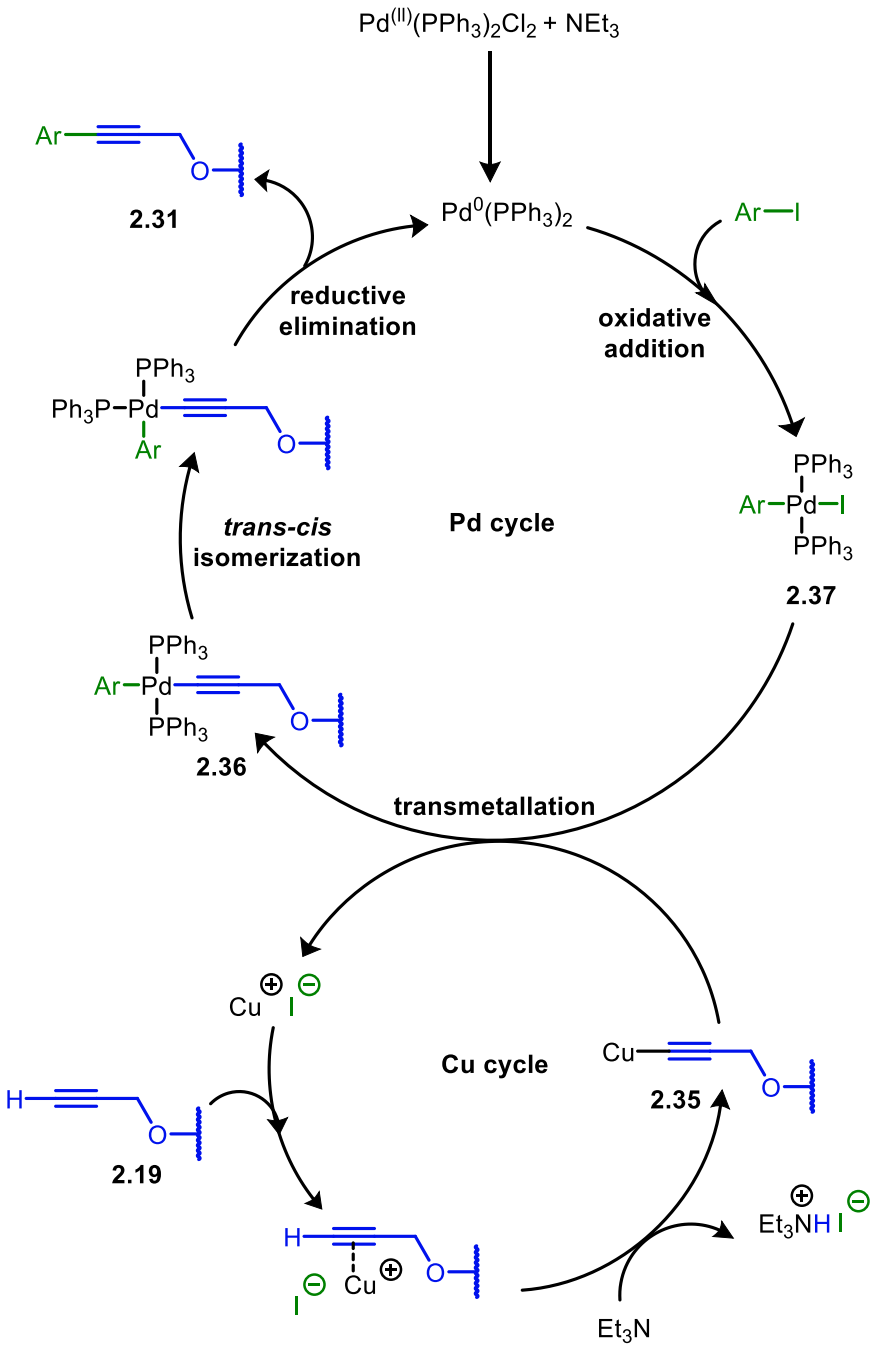


Figure 2.8: General Sonogashira catalytic cycle.

Following Sonogashira cross coupling, the tertiary amine products **2.31** were oxidised with *m*-CPBA in DCM leading to the *N*-oxide intermediate **2.32**. These immediately underwent Cope elimination of the cyanoethyl group giving the hydroxylamine **2.33** *in situ*. Reverse-Cope cyclisation of these alkyne-hydroxylamines afforded the enamine *N*-oxide species **2.34**, which were all obtained as single diastereomers, as confirmed by ^{13}C NMR. The geometry about the enamine double bond was also fixed

as the *trans*-isomer due to the concerted *syn* mechanism of the reverse-Cope reaction. A characteristic enamine proton was observed *ca.* 8.20 ppm in the ^{1}H NMR, which is substantially higher than where you would expect to see an enamine signal, caused by the inductive effect of the nearby positive *N*-oxide nitrogen, shifting the signal downfield.

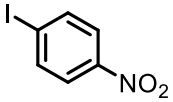
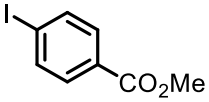
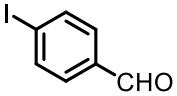
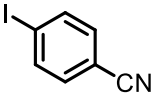
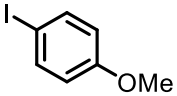
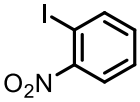
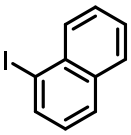
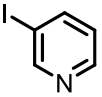
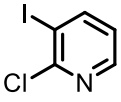
Entry	Aryl Iodide	2.31 Yield (%)	2.34 Yield (%)
1		**70**	**86**
2		**95**	**83**
3		**52**	**87**
4		**68**	**88**
5		**72**	**N/R**
6		**73**	**84**
7		**99**	**47**
8		**83**	**47**
9		**90**	**50**

Table 2.1: Sonogashira and oxidation yields with varying aryl iodides.

The *p*-methoxy phenyl analogue of **2.31.5** was the only example which did not successfully undergo tandem Cope elimination/reverse- Cope cyclisation. This was attributed to the electron donating character of the methoxy group, compared to the electron withdrawing substituents in all other examples. The electron withdrawing groups activate the alkyne to attack by both inductive and mesomeric effects (Figure 2.9). Conversely, the electron donating methoxy group acts in the opposite way causing the reverse-Cope cyclisation reaction to be shut down.

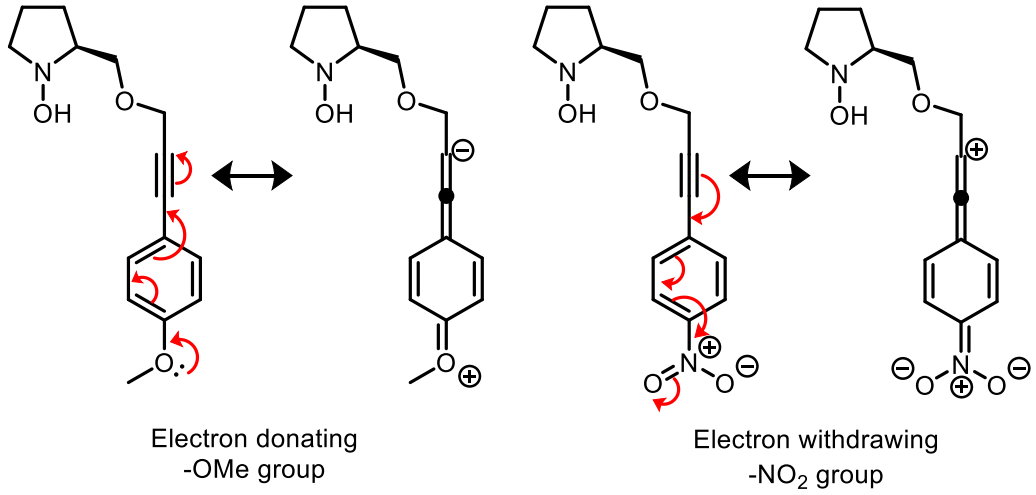
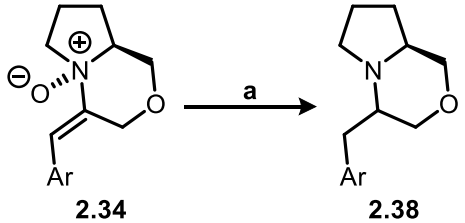


Figure 2.9: Electron donating and withdrawing group effects on reverse-Cope cyclisation.

Three of the enamine *N*-oxide **2.34** analogues, naphthyl, *p*-cyanophenyl and *p*-methyl benzoate were globally reduced to their parent amine species **2.38** by catalytic hydrogenation using palladium on carbon in methanol (Scheme 2.16). Hydrogenation of all three substrates (Table 2.2), occurred with complete diastereoselectivity as the corresponding hydroxymethyl derivative **2.29** had (Section 2.4.4.). This was once again confirmed by ^{13}C NMR.



2.34 **2.38**

Scheme 2.16: Hydrogenation of enamine *N*-oxide **2.34**. *Reagents and Conditions* **a-** H_2, Pd/C (0.1 eq.), MeOH, rt, o/n.

Reduction of the *p*-cyanophenyl analogue **2.38.1** was significantly lower yielding than that of the other two examples, due to hydrogenation of the nitrile group, leading to a complex mixture of products, including the benzyl amine and toluene analogues

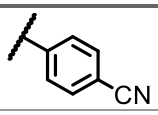
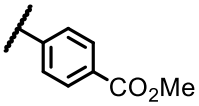
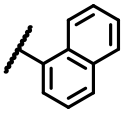
Entry	Ar	2.38 Yield (%)
1		6
2		58
3		86

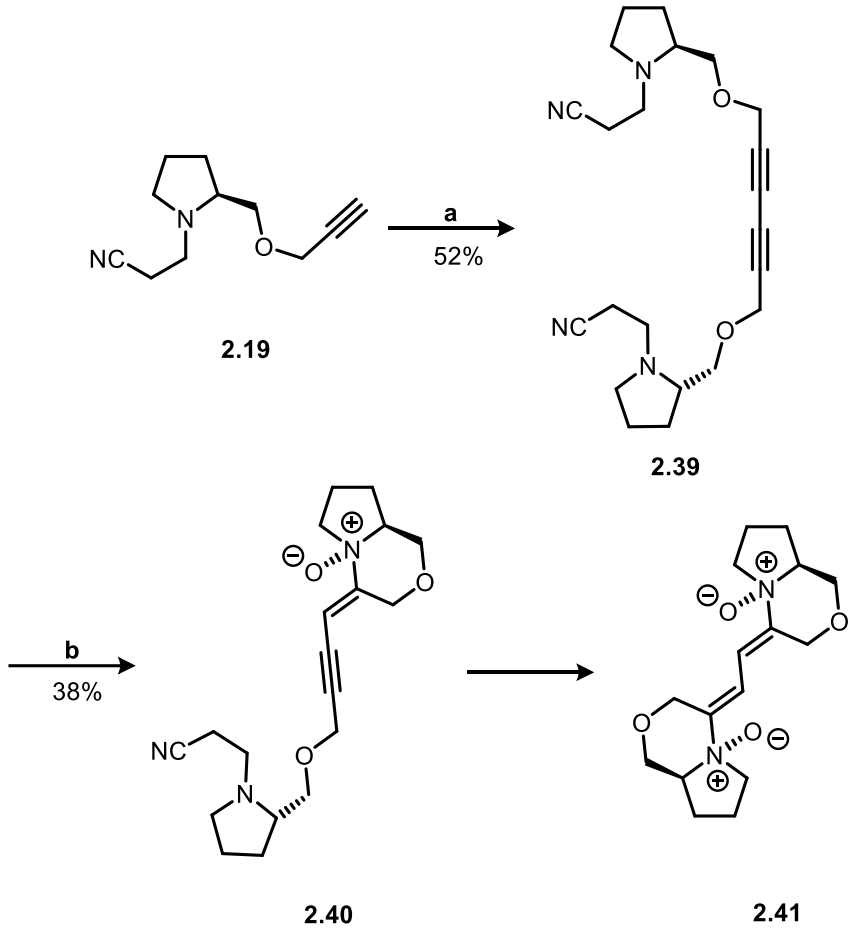
Table 2.2: Yields of enamine *N*-oxide hydrogenations.

2.4.6. Synthesis of Bis-Enamine *N*-Oxide

We also wished to investigate the synthesis of dimeric enamine *N*-oxide species such as compound **2.41**, shown in Figure 2.17, as the potential properties of these compounds are of interest. Alkyne **2.19** was dimerised in Glaser coupling using CuI as the catalyst, under an oxygen atmosphere, which gave the bis-amine **2.39** in a 52% yield. The loss of the terminal alkyne proton at 2.43 ppm in the ^{1}H spectrum was observed upon conversion to the bis-amine. Because of the symmetrical nature of the product only one set of signals was observed.

The amine dimer **2.39** was then oxidised using *m*-CPBA in DCM, containing a K_2CO_3 buffer, and after 1 day at ambient temperature had undergone monocyclisation. The single oxidation was attributed to the highly polar nature of *N*-oxide **2.40** causing it to precipitate out of solution, and so oxidation of the second amine did not occur. To force the reaction to completion it was heated to reflux overnight, which initiated the second oxidation and Cope elimination/reverse-Cope cyclisation, yielding the bis-enamine *N*-oxide species **2.41** in a 53% yield. The product was obtained as a highly

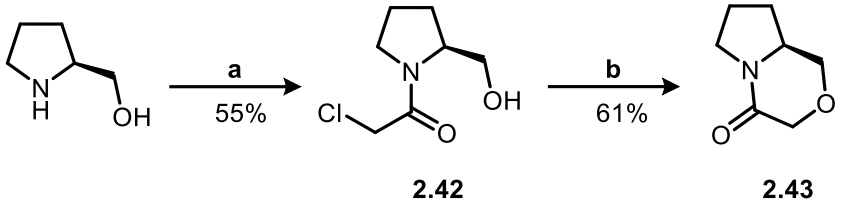
stable powder, which was identified by the distinct enamine protons, which were observed at 7.53 ppm, as well as loss of the cyanoethyl group of **2.19** in the ^{1}H NMR.



Scheme 2.17: Synthesis of bis-enamine *N*-oxide. *Reagents and Conditions* **a-** TMEDA (0.4 eq.), CuI (0.20 eq.), O_2, DCM, o/n. **b-** *m*-CPBA (2.4 eq.), K_2CO_3 (2.5 eq.), DCM, -78°C - reflux, 2 days.

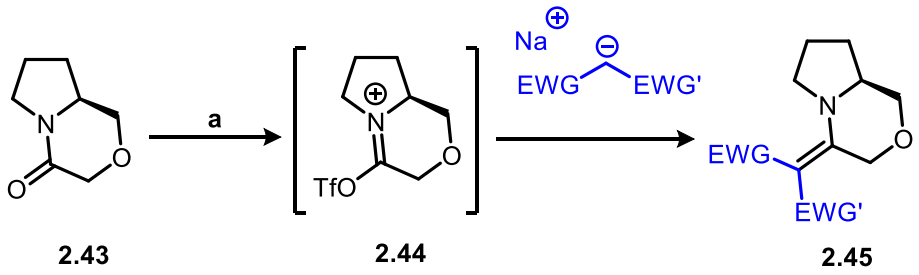
2.4.7. Route to Template *via* Bicyclic Lactam Structure

A condensation reaction of lactam **2.43** (Scheme 2.18), which was reported in a 1986 publication by Roark *et al.*, was proposed as an alternative method of functionalisation at the *α*-position to the nitrogen of this bicyclic system.[124] Synthesis of lactam **2.43** was performed following the same procedure as that described in their work. Prolinol **2.5** was treated with chloroacetyl chloride in a buffered solution of sodium acetate, acetone and water to give the *α*-chloroamide **2.42** in a 55% yield. Deprotonation of **2.42** in THF triggered lactam ring closure, affording **2.43** in a 61% yield as an off white solid.



Scheme 2.18: Synthesis of bicyclic lactam. *Reagents and Conditions* **a-** $MeCO_2Na$ (2.0 eq.), $ClCH_2COCl$ (1.0 eq.), acetone, H_2O, 0°C, 2h **b-** NaH (1.3 eq.), THF, o/n.

A mild Knoevenagel-type lactam condensation reaction, which was described in a 2014 publication by Wang and co-workers, was utilised for reduction of lactam **2.43**.[125] Lactam **2.43** was first activated to the iminium triflate intermediate **2.44** using triflic anhydride in DCM at -78 °C (Scheme 2.19). The iminium triflate **2.44** was then trapped out by addition of carbanions, which were pre-formed separately by deprotonation with NaHMDS, to yield the conjugate enamine product **2.45**.



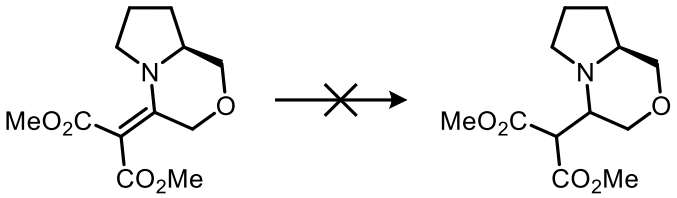
Scheme 2.19: Synthesis of bicyclic lactam. *Reagents and Conditions* **a-** NaHMDS (2.2 eq.), Tf_2O (1.1 eq.), DCM, -78°C, o/n.

The yields of all lactam condensations reactions are summarised in table 2.3 below. When EWG=EWG' (entries 1 and 2) the reaction proceeded to give the conjugated enamine species in reasonable yields, which were identified by HRMS. There was no product isolated from the reaction of the iminium triflate and the carbanions of either nitromethane or ethyl bromoacetate which was surprising given the fact that corresponding examples had been reported in the publication by Wang and co-workers.

Entry	EWG	EWG'	2.45 Yield (%)
1	CO_2Me	CO_2Me	63
2	CN	CN	37
3	NO_2	H	0
4	CO_2Et	H	0

Table 2.3: Yields of Knoevenagel-type lactam condensation reaction.

Attempts to reduce the enaminone **2.45** double bond, shown in Scheme 2.20, by catalytic hydrogenation are summarised in Table 2.3. No tertiary amine **2.46** was isolated in any of the examples shown, however despite the fact hydrogenation of similar systems has been shown to proceed under the conditions depicted in entries 2 and 3.[126] It was unclear why the double bond could not be reduced in this case compared to those shown to be successful in the literature. One possible explanation is that the high degree of conjugation within the system of **2.45** delocalises the electron density away from the enamine double bond, preventing reduction being achieved. However, no experimental evidence could be provided for this theory.



2.45 **2.46**

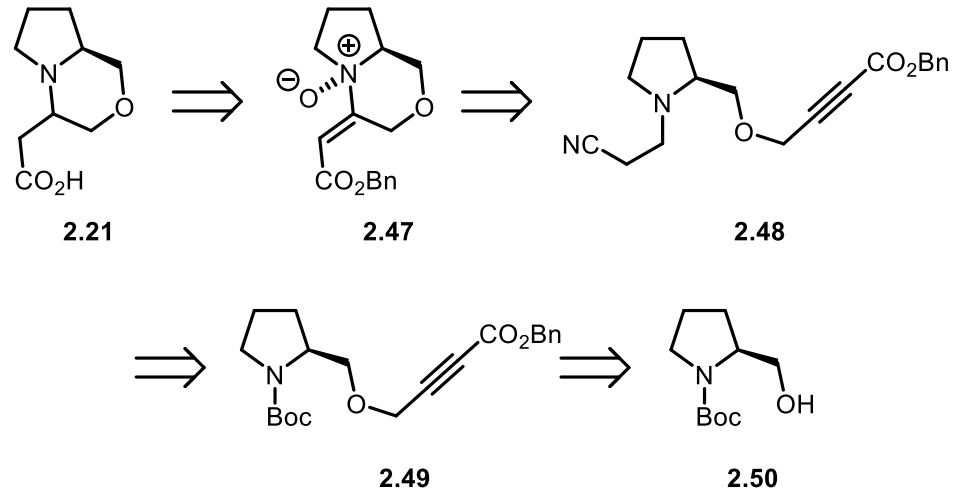
Scheme 2.20: Attempted enaminone reduction.

Entry	Catalyst	Equivalents	Solvent	Pressure (Bar)	Temperature (°C)	Yield %
1	Pd/C	0.1	MeOH	1	25	0
2	Pd/C	0.1	MeOH	5	25	0
3	Pd/C	0.1	MeOH	5	60	0
4	PtO_2	0.05	MeOH	1	25	0

Table 2.4: Attempted enaminone reduction methods

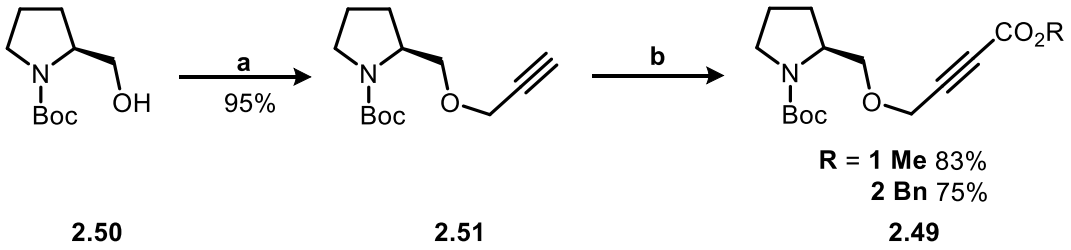
2.4.8. Alternative Route Towards Bicyclic Morpholine System

As discussed in Section 2.4.3. the deprotonation of the terminal alkyne proton of **2.19**, in order to alkylate at this position, was hindered by a competitive *β*-elimination of acrylonitrile taking place preferentially. The retrosynthetic route depicted in Scheme 2.21 was envisaged to circumvent this competitive reaction pathway. The functionalised alkyne **2.48** would be synthesised from Boc-*L*-prolinol **2.50**, by propargylation of the alcohol followed by *C*-acylation. The amine could then be deprotected and the *N*-cyanoethyl group introduced to give the key intermediate **2.49**. This would then undergo the tandem Cope elimination/reverse-Cope cyclisation reaction to give the conjugated enamine *N*-oxide **2.47**. Finally, global hydrogenation of **2.47** would give the bicyclic morpholine template **2.21** with the required acid functionalisation. Whilst this synthetic route is longer that desired, this was outweighed by the necessity of reaching the key acid functionalised template **2.21** required for *β*-turn mimetic synthesis.



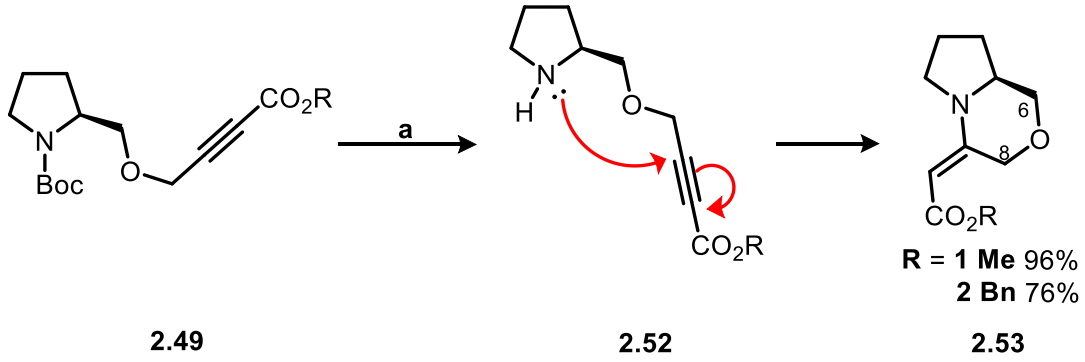
Scheme 2.21: Proposed retrosynthetic route to acid functionalised morpholine

Boc-*L*-prolinol was deprotonated with sodium hydride in THF at 0°C followed by addition of propargyl bromide to give **2.51** in a 95% yield (Scheme 2.22). The terminal alkyne was then deprotonated with *n*-BuLi in THF to give the lithium acetylide *in situ*. This was trapped out with methyl and benzyl chloroformates to give the propargylic esters **2.49** in 83% and 75% yields respectively. In both cases the loss of the alkyne CH proton signal at 2.42 ppm was observed. The introduction of methyl singlet at 3.78 ppm and benzylic CH_2 singlet at 5.20 ppm was also seen for the relevant propargylic esters.



Scheme 2.22: Alkyne functionalisation. *Reagents and Conditions* **a-** NaH (1.2 eq.), propargyl bromide (1.1 eq.), THF, 0 °C, o/n. **b-** *n*-BuLi (1.1 eq.), $ClCO_2R$ (1.1 eq.), THF, -78 °C, o/n.

With the Boc-protected amines **2.49** in hand, Boc-deprotection followed by reaction with acrylonitrile was required to give tertiary amine **2.48**. The Boc-protected amines **2.49** were treated with an excess of TFA in DCM, the pH of the solution was then adjusted to 7-8 with 2M NaOH to free base the amines (Scheme 2.23). The isolated product was significantly less polar by TLC than expected and also did not have the characteristic streak that amines exhibit. The CH_2 proton signals of carbons 6 and 8 had also split into distinct AB quartets (Figure 2.10), indicative of hindered rotation about these points. All this evidence suggested that a serendipitous cyclisation of the amines **2.52** to the *β*-enamino esters **2.53** had occurred. This ring closing reaction is a favoured 6-*exo*-*dig* process, which is favourable according to Baldwin's rules.[127, 128]



Scheme 2.23: Bicyclic enamino ester template synthesis. *Reagents and Conditions* **a-** (i) TFA, DCM, 0 °C, 3h (ii) 2M NaOH.

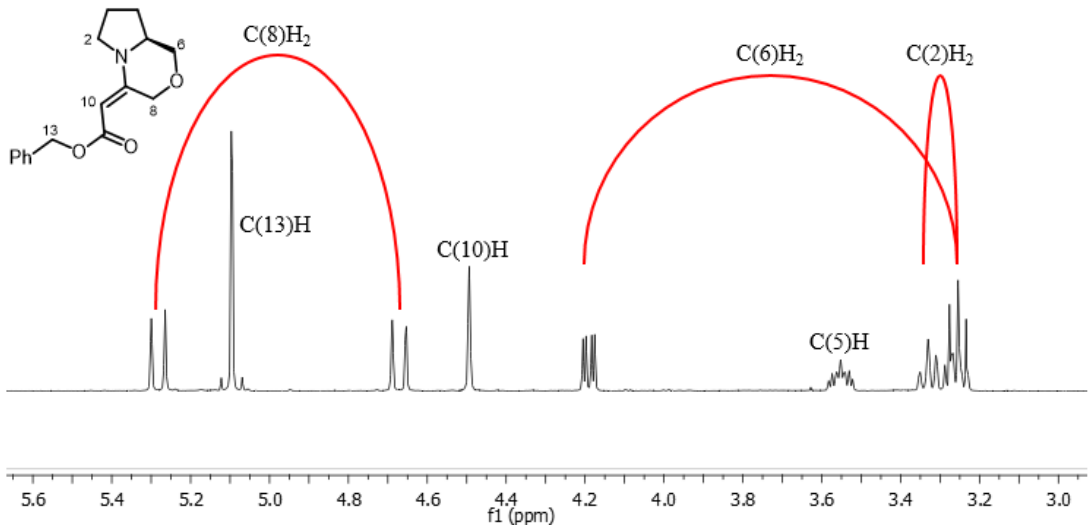


Figure 2.10: NMR spectra of *β*-enamino benzyl ester showing AB quartets.

The geometry about the enamine double bond was elucidated by nOe experiments, irradiating the enamine proton H^a, shown in red in Figure 2.11. A through space interaction was observed between the enamine proton and the two pyrrolidine ring protons H^b, shown in green, confirming *trans* geometry about the bond.

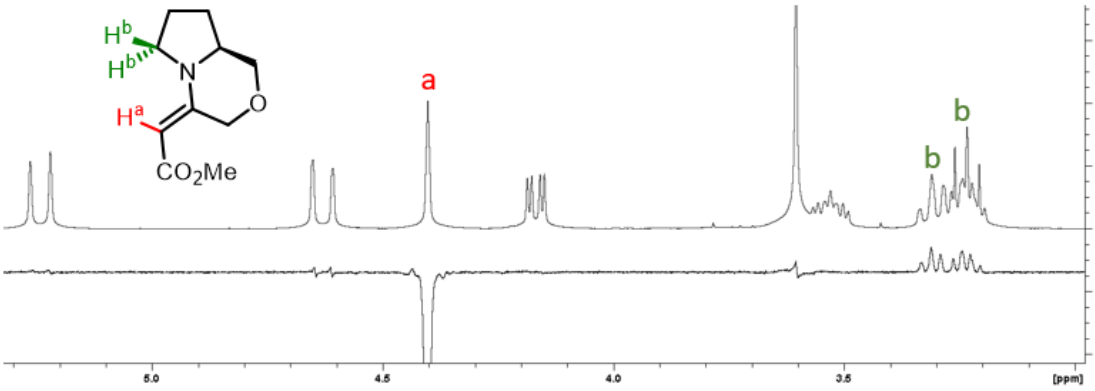
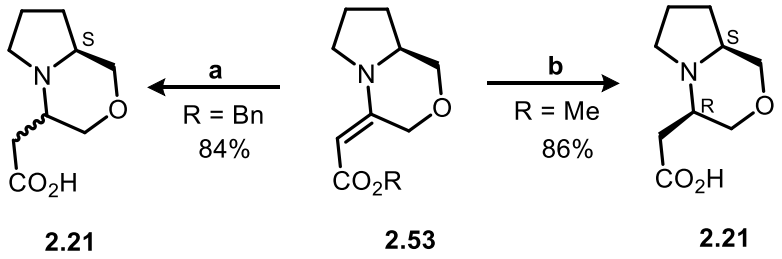


Figure 2.11: nOe spectra of *β*-enamino methyl ester showing through space interaction of enamine proton and pyrrolidine ring CH_2.

The unforeseen cyclisation to the *β*-enamino esters **2.53** reduced the length of the synthetic route, removing the need to carry out amine alkylation and tandem Cope elimination/reverse Cope cyclisation, to the bicyclic system. The *β*-enamino esters were then reduced to the bicyclic morpholine **2.21**, shown in Scheme 2.24. The *β*-

enamino benzyl ester **2.53.2** was globally reduced to **2.21** by catalytic hydrogenation with palladium on carbon in methanol. Whereas, the methyl ester analogue **2.53.1** underwent catalytic hydrogenation using palladium on carbon, which was then followed by ester hydrolysis to give **2.21**.



Scheme 2.24: Synthesis of carboxylic acid functionalised bicyclic templates. *Reagents and Conditions* **a-** H_2, Pd/C (0.1 eq.), MeOH, rt, o/n. **b-** (i) H_2, Pd/C (0.1 eq.), MeOH, rt, o/n (ii) LiOH (1.1 eq.), MeOH, H_2O, 3h.

β-Enamino ester reduction to morpholine **2.21** was high yielding in both cases. The key difference between the two routes was the diastereoselectivity of the hydrogenation. Global hydrogenation of the *β*-enamino benzyl ester gave a 4:1 mixture of diastereoisomers. In contrast, hydrogenation of the methyl ester analogue proceeded with complete diastereoselectivity. The product was obtained as a single spot by TLC and no doubling of signals was observed in the ^{13}C NMR. Hydrogenation was confirmed to have given exclusively the *S,R*-diastereoisomer of the bicyclic morpholine **2.21**. This was confirmed by single X-ray crystallography of a subsequent compound synthesised from the **2.55.1** (Section 2.4.9.). By analogy between the morpholine products of each reduction route it was confirmed that the major product in the global *β*-enamino benzyl ester reduction was also the *S,R*-diastereomer. The reason for the complete selectivity of double bond reduction was not immediately apparent. One possible reason is due to the shape of the structure. The lowest energy conformation of the *β*-enamino methyl ester **2.53.1** was calculated, using the ωB97X-D functional and 6-31G* basis set, which is shown in Figure 2.12. This structure suggested that there was a concave surface on the top face of the template. This could cause catalytic hydrogenation on the bottom face to be favoured, leading to the *S,R*-diastereoisomer.

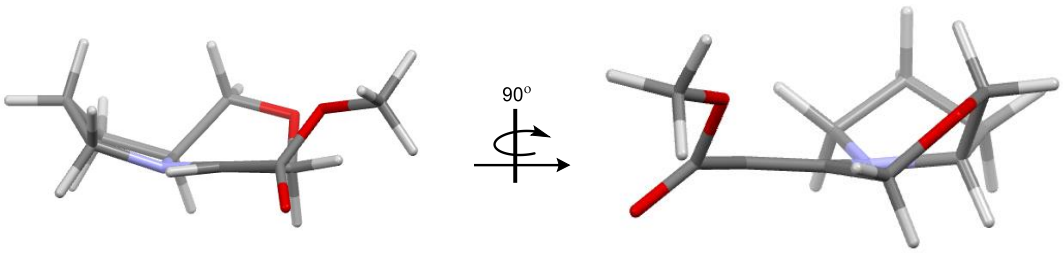


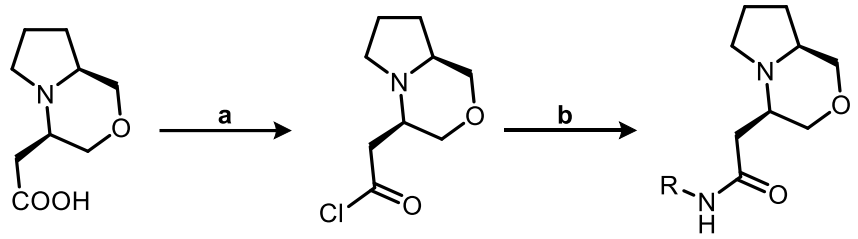
Figure 2.12: *β*-enamino methyl ester **2.53.1** lowest energy conformation shown looking down (left) and across (right) the enamine double bond.

Due to the diastereoselectivity of the route *via* the methyl ester analogue, this was used for all future work towards the *β*-turn mimetic compounds. The benzyl ester analogue **2.53.2** could be used in the future as a route into the *S,S*-diastereomer if required. This route successfully synthesised the key acid functionalised morpholine from Boc-prolinol in a 65% yield over 5 steps. It is also worth noting that this route was scaled up to a multigram scale (*ca.* 15 g Boc-prolinol) with little loss in yield.

2.4.9. Amide Coupling Reactions to Morpholine Acid Handle

It was decided that amide coupling reactions could be carried out on the fused morpholine acid template *via* the acyl chloride **2.54** (Scheme 2.25). The primary reason for carrying out the amide couplings this way was to avoid the by-products made when using amide coupling reagents such as HATU or EDC. Test reactions with these amide coupling reagents were carried out on small scales. These found that due to the polar nature of both the tertiary amine containing product and HATU/EDC by-products purification was troublesome. Aqueous work up to remove impurities was not possible as significant amounts of the desired product was also lost. Also, the chiral centre would not racemize due to acyl chloride formation, a common drawback of using this method.

Acid **2.21** was treated with oxalyl chloride and a catalytic amount of DMF, which reacted with the acid to give acyl chloride **2.54**. Once excess oxalyl chloride was removed, the acid chloride **2.54** was trapped out with a number of amines to give the bicyclic morpholines **2.55**. Yields of individual amide coupling reactions are summarised in Table 2.5. Amides **2.55** synthesis was confirmed by HRMS.



2.21 **2.54** **2.55**

Scheme 2.25: Miscellaneous amide couplings with acid functionalised bicyclic morpholine **X**. *Reagents and Conditions* **a-** $(COCl)_2$ (1.5 eq.), DMF (cat.) DCM, 0 °C **b-** RCH_2NH_2 (1.0 eq.), DIPEA (1.0 eq.).

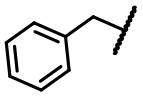
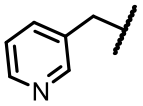
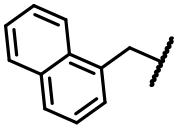
Entry	R	2.55 Yield (%)
1		69
2		63
3		59

Table 2.5: Amide coupling reaction yields.

These amides were isolated as either powders or viscous oils, all of which were stable for prolonged periods of time when stored in the freezer. After recrystallization of **2.55.1** a crystal structure was obtained, shown in Figure 2.13, which confirmed the diastereoselectivity of the *β*-enamino methyl ester **2.53.1** hydrogenation step (Section 2.4.8).

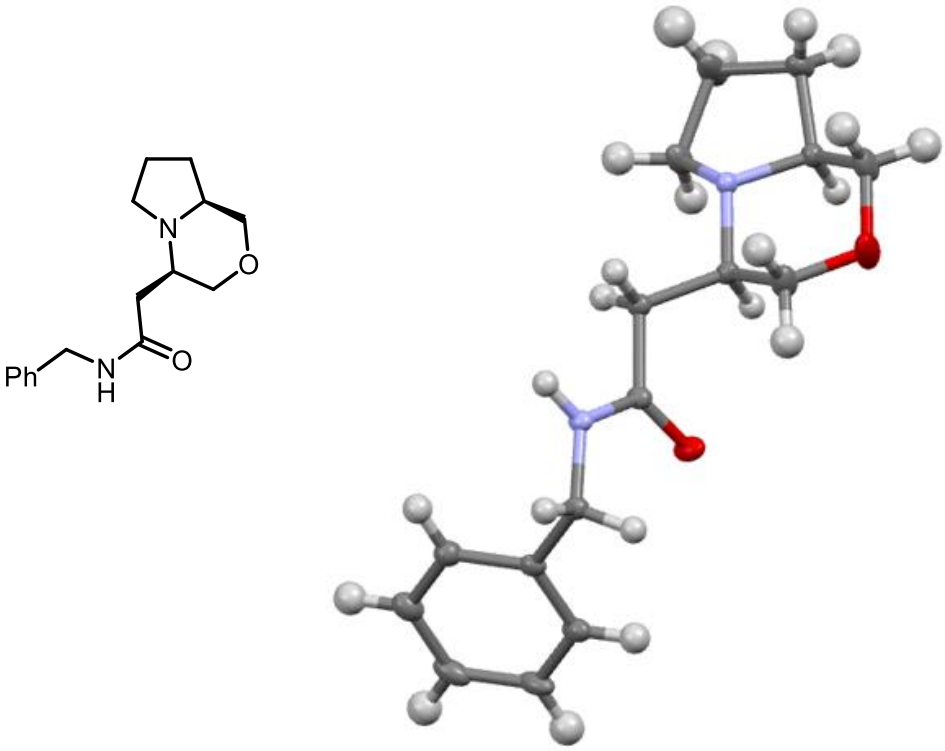
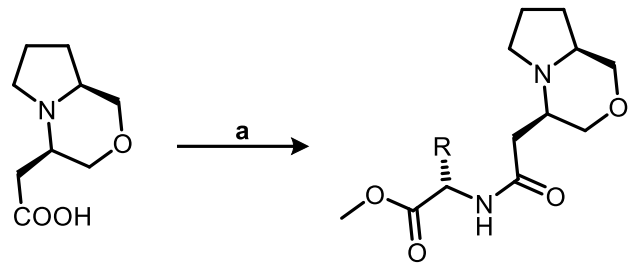


Figure 2.13: Crystal structure of bicyclic morpholine benzyl amide analogue **2.55.1**.

Having established a method for amide coupling of **2.21** with a series of amines, focus moved to compounds requested by LifeArc for testing within their active PPI project. A number of suitably protected amino acids were coupled, using the same method as described for compounds **2.55**, to the bicyclic morpholine **2.56** (Scheme 2.26). The protected amino acids chosen were the protected versions of those which molecular modelling suggested could interact best with the protein target. Protected amino acid hydrochlorides were used for these reactions, so an extra equivalent of DIPEA was also added to the reaction to free base the amino acids in the reaction mixture. All amino acids were used as the methyl esters. Therefore, as well as HRMS, the appearance of a methyl ester singlet, ca. 3.74 ppm, observed in the ^{1}H spectrum of each of the examples confirmed successful coupling.



2.21 **2.56**

Scheme 2.26: Amide couplings with acid functionalised bicyclic morpholine **2.21**. *Reagents and Conditions* **a-** (i) $(COCl)_2$ (1.5 eq.), DMF (cat.) DCM, 0 °C (ii) AA.HCl (1.0 eq.), DIPEA (2.0 eq.).

The yields of the protect amino acid amide couplings are summarised in Table 2.6. Yields were corresponded to those of the simpler amide couplings, Table 2.6. The amides **2.56** were isolated as a mixture of powders and viscous oils. They were all stable for prolonged periods of time if stored well sealed. Over time they were all slightly hygroscopic, but little to no decomposition was observed.

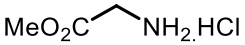
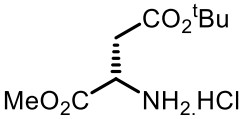
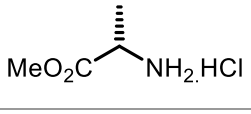
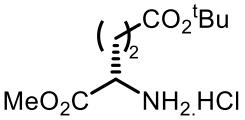
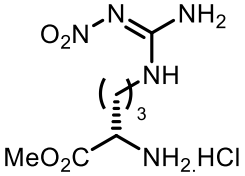
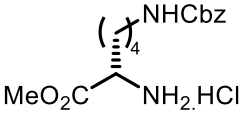
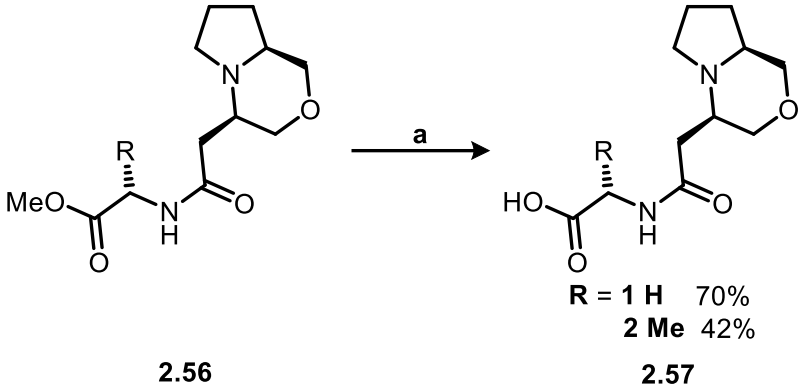
Entry	AA.HCl	2.56 Yield (%)	Entry	AA.HCl	2.56 Yield (%)
1		68	**4**		67
2		64	**5**		74
3		48	**6**		92

Table 2.6: Protected amino acid amide coupling reaction yields.

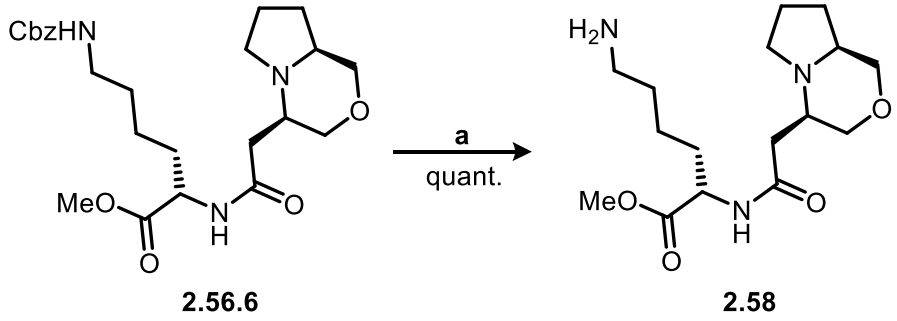
2.4.9.1 Amino Acid Deprotections

Methyl esters **2.56.1 and 2.56.2** were hydrolysed to the corresponding acids **2.57** by treatment with lithium hydroxide in MeOH, as shown in Scheme 2.27. To isolate **2.57** the reaction mixture was adjusted to pH 5-6 with 2M HCl to give the zwitterion species, followed by column chromatography during which the carboxylic acid was protonated on the silica gel. Care was taken as lowering the pH too much would result in protonation of the tertiary amine. Methyl ester hydrolysis was carried out on the Ala and Gly analogues only, mainly due to time constraints on compound submission for SPR testing.



Scheme 2.27: Ester hydrolysis of protected amino acid residues. *Reagents and Conditions* **a-** (i) LiOH (1.1 eq.), MeOH, H_2O, rt, 3h (ii) 2M HCl.

The Cbz protecting group of the lysine analogue **2.56.6** was also removed by catalytic hydrogenation with palladium on carbon in methanol. The reaction was followed to completion by the disappearance of the starting material and the emergence of the highly polar amine product. Deprotection proceeded to give the amine **2.58** in quantitative yield.



Scheme 2.28: Cbz-lysine side chain deprotection. *Reagents and Conditions* **a-** H_2, Pd/C (0.1 eq.), MeOH, rt.

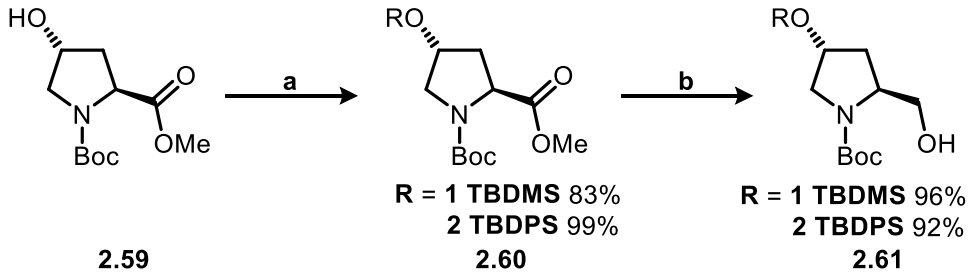
2.4.10. Incorporation of Additional Functionality Template Handles

To develop the bicyclic morpholine template, discussed in this chapter, we wished to introduce additional handles to the system. These could be used to further expand the scope of this scaffold in the future. The greater the possible variations the template can offer the more flexible it becomes as a potential source of drug like compounds.

2.4.10.1. 4-Hydroxyl-*L*-Proline Derived Morpholine Template

It was thought that a hydroxyl group could be introduced onto the pyrrolidine ring of the bicyclic system. The synthesis of these analogues would start from the protected 4-hydroxyproline **2.59**. Hydroxyproline derivatives are the basis of a variety of therapeutics, just a few of which were summarised in a review by Bach and co-workers in 2013.[129] Introduction of the hydroxyl group onto the pyrrolidine ring system could also allow for increased β-turn receptor affinity, as the ring itself is acting as a β-turn scaffold.

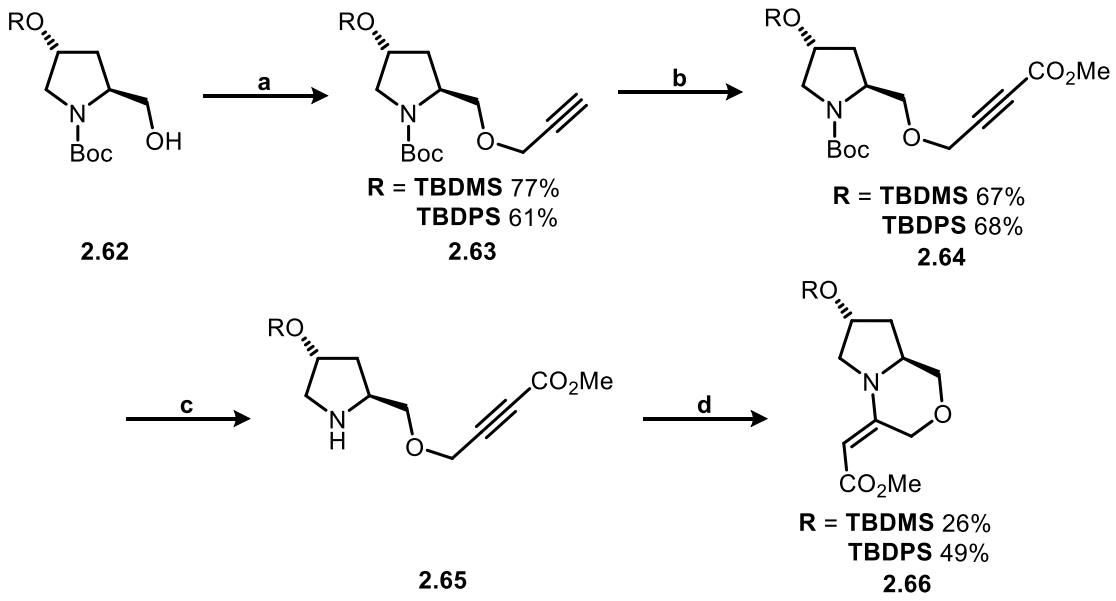
Following a literature procedure the *N*-protected 4-hydroxy-*L*-proline **2.59** was *O*-silyl protected by treatment with the respective silyl chloride and excess imidazole as base (Scheme 2.29).[130] This gave the globally protected 4-hydroxy-*L*-proline derivatives **2.60** in high yields. Methyl ester reduction was then carried out using lithium borohydride in THF to yield the functionalised Boc-prolinol derivatives **2.61** in excellent yields.



Scheme 2.29: Synthesis of 4-hydroxy-Boc-prolinol. *Reagents and Conditions* **a-** silyl chloride (1.2 eq.), imidazole (2.2 eq.) DMF **b-** $LiBH_4$ (1.5 eq.), THF, 0°C, o/n.

With **2.61** in hand, possessing suitably protected 4-hydroxyl groups, the same synthetic strategy could be employed as previously in Section 2.4.8 to make the

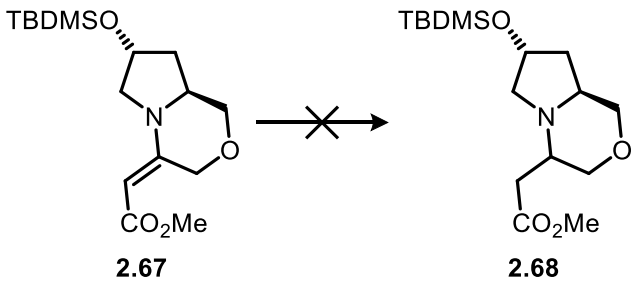
bicyclic morpholine (Scheme 2.30). Williamson ether synthesis was achieved by hydroxyl deprotonation using sodium hydride followed by addition of propargyl bromide in THF, which gave the alkyne species **2.63** which was confirmed by HRMS. Terminal alkyne deprotonation with *n*-BuLi generated the active carbolithium intermediate, which upon addition of methyl chloroformate reacted to give the propargylic ester **2.64** in reasonable yields. Boc deprotection was achieved by treatment of **2.64** with TFA in DCM to give the corresponding amine **2.65**, which unlike previous derivatives did not spontaneously cyclise to the *β*-enamino ester **2.66**. Heating **2.65** in methanol induced cyclisation, which was monitored by TLC for loss of the more polar amine spot to the to the *β*-enamino ester **2.66** in relatively low yields after column purification.



Scheme 2.30: Synthesis of 4-hydroxy bicyclic *β*-enamino ester. *Reagents and Conditions* **a-** (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) propargyl bromide (1.2 eq.) **b-** (i) *n*-BuLi (1.1 eq.), THF, -78°C, 40 mins (ii) $ClCO_2Me$ (1.1 eq.), o/n **c-** TFA, DCM **d-** MeOH, reflux o/n.

Hydrogenation of the *β*-enamino ester **2.66** was attempted using palladium on carbon in methanol, the same conditions that had been applied to the corresponding derivative lacking the 4-hydroxyl group (Section 2.4.8.). However, no conversion to the tertiary amine **2.68** was observed on this occasion and only starting material was recovered from the reaction mixture. From the results discussed in Section 2.4.8. hydrogenation

from the bottom face of these bicyclic systems is heavily favoured, which would suggest that the presence of a very bulky silyl protecting group may be responsible for no reaction of starting material in this case.



2.67 **2.68**

Scheme 3.31: Attempted hydrogenation of *β*-enamino ester **2.67**. *Reagents and Conditions* **a-** H_2, Pd/C (0.1 eq.), MeOH, o/n.

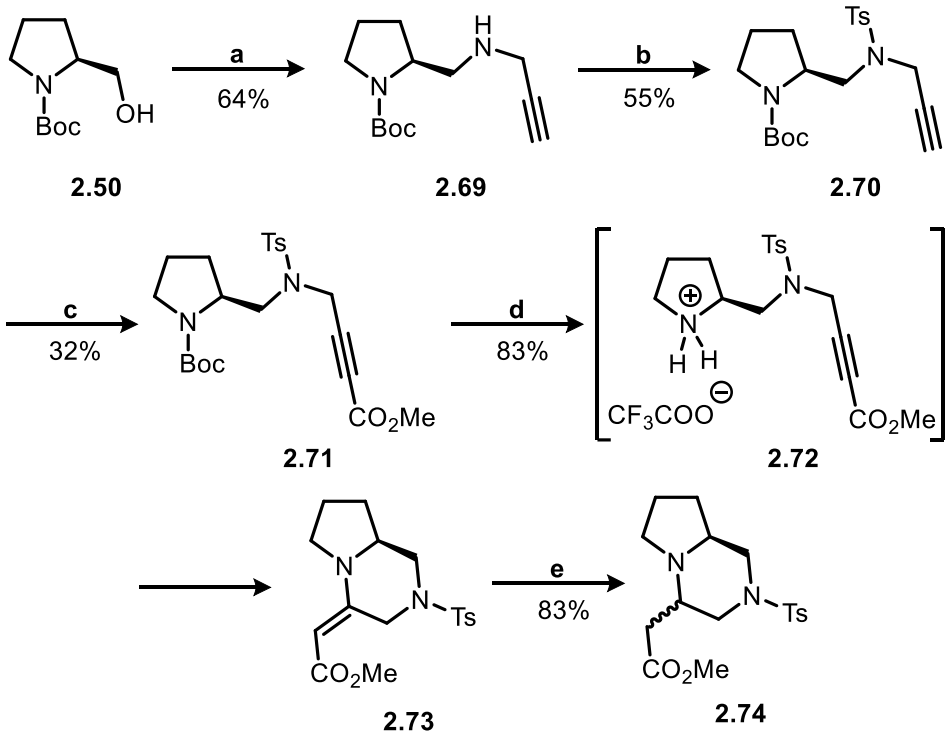
Deprotection of the silyl protecting group prior to reduction of the *β*-enamino ester could potentially circumvent this problem, achieving the addition of the hydroxyl handle to the bicyclic morpholine template. This was not carried out in this work due to time constraints, but would be prioritised in the future.

2.4.10.2. Bicyclic Piperazine Ring Derivative

Another avenue of investigation was the synthesis of a bicyclic piperazine ring system, which was analogous to the previously described morpholine systems. By substitution of the morpholine for piperazine ring, within the bicyclic structure, the additional nitrogen could act as another point by which the compound could be diversified. This position of this extra nitrogen also sits at a point in the compound which could act as the turn component of a mimetic, making it a potential extra site for a protein-inhibitor interaction.

The bicyclic piperazine derivative was synthesised using a similar route to the corresponding morpholines, shown in Scheme 2.32. Boc-prolinol **2.50** was oxidised to Boc-prolinal using catalytic TEMPO in the presence of trichloroisocyanuric acid. The aldehyde underwent *in situ* reductive amination with propargyl amine to afford **2.69** in a 64% yield. Due to observations made, which are discussed later, the amine side chain of **2.69** was then tosyl protected by treatment of **2.69** with tosyl chloride and base to give the protected amine **2.70**, which was significantly less polar than the

amine starting material and exhibited a distinctive sulfonamide stretch at 1347 cm^{-1} in the IR spectrum.

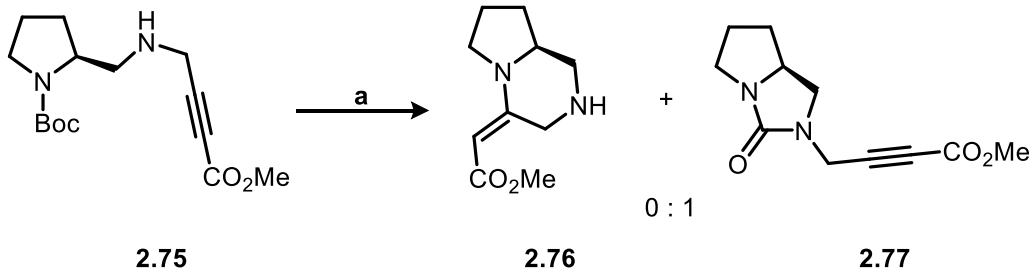


Scheme 2.32: Synthetic route towards bicyclic piperazine ring derivative. *Reagents and Conditions* **a-** (i) TEMPO (0.01 eq.), trichloroisocyanuric acid (1.1 eq.), DCM, 0°C, 20 mins (ii) Propargyl amine (1.5 eq.), STAB (5.0 eq.), MeOH, 0°C, o/n **b-** TsCl (1.2 eq.), NEt_3 (1.5 eq.), DCM, 0°C, o/n **c-** *n*-BuLi (1.1 eq.), $ClCO_2Me$ (1.1 eq.), THF, -78°C, o/n **d-** TFA, DCM, 0°C **e-** H_2, Pd/C (0.1 eq.), MeOH.

The terminal alkyne of the protected amine **2.70** was deprotonated using *n*-BuLi in THF, after which the carbanion was *C*-acylated with methyl chloroformate to give the propargylic ester **2.71** in a 32% yield. Boc-deprotection of **2.71** using TFA in DCM gave the trifluoroacetate ammonium salt **2.72** *in situ*, which upon treatment with base using 2M NaOH spontaneously cyclised to give the *β*-enamino ester **2.73** in a 83% yield. The product exhibited a definitive enamine singlet in the ^1H NMR at 4.33 ppm as well as the characteristic AB quartet splitting of both piperazine ring CH_2 groups. Hydrogenation of the *β*-enamino ester **2.73** with palladium on carbon in methanol gave the corresponding tertiary amine **2.74** in an 83% yield as a 1:1 inseparable mixture of diastereoisomers. This was somewhat surprising considering the complete selectivity

of the morpholine derivative *β*-enamino ester reduction, although the tosyl protected amine bulk would almost certainly alter the overall bicyclic structure, leading to these results.

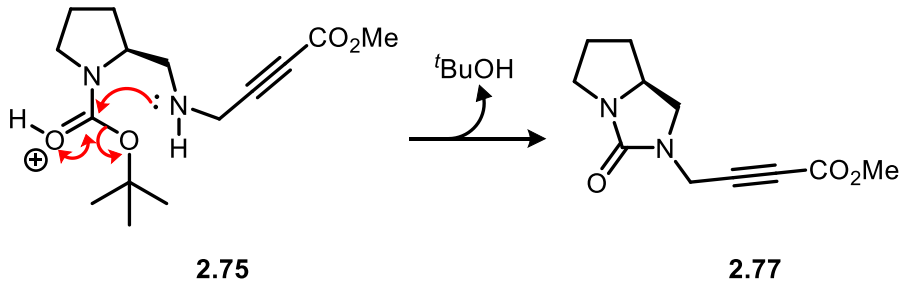
The requirement of a tosyl protecting group on the amine contained in the pyrrolidine side chain was realised when attempted cyclisation was made with the free amine. Reaction of **2.75** with TFA in DCM yielded a single product, which upon closer inspection became clear was not the desired *β*-enamino ester **2.77**. Specifically, no amine spot was seen on the TLC of the product as well as the presence of two carbonyl peaks in the carbon NMR, which is not possible if the isolated product was **2.76**. The unexpected product was identified by 2D NMR elucidation, and later confirmed by mass spectrometry.



Scheme 3.34: Boc-deprotection in the presence of free amine side chain.

Reagents and Conditions **a-** TFA, DCM, 0°C.

The likely mechanism of the urea formation which occurred upon addition of TFA to the Boc-protected amine **2.75,** shown in Scheme 2.35, proceeds by amine addition into the protonated Boc group loosing *t*-butanol to yield **2.77**.



Scheme 2.35: Proposed mechanism of urea side product formation

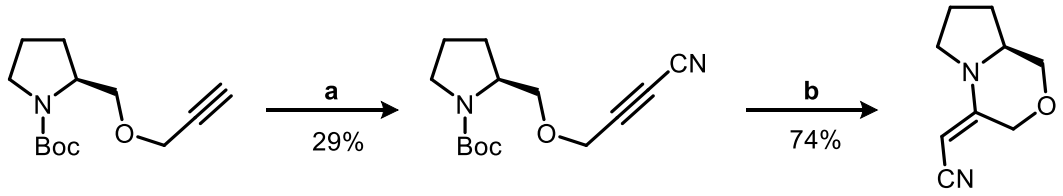
The tosyl group was used as it would remain stable under the conditions required for the synthesis of the bicyclic template. Other groups such as Boc or Cbz would be also be removed under the acidic conditions of pyrrolidine Boc-deprotection. This

protecting group could be chemoselectively removed when required, using reagents such as a Li/naphthalene. The free amine of the piperazine could then act as a further point of diversification of the bicyclic system, increasing the synthetic flexibility of the template.

2.4.10.3. *β*-Turn Mimetic Reversal by Introduction of Morpholine Amine Handle

One final point of exploration into the further modification of the bicyclic morpholine template was the chain reversal of the *β*-turn mimetic, achieved by positioning the *N*-terminus on the morpholine ring instead of the *C*-terminus. It was decided that a nitrile group would be used as the source of the protected amine the electron withdrawing nature of the group would drive the intramolecular cyclisation post Boc-deprotection. The nitrile would also provide an alternative route into the *C*-terminus derivative by hydrolysis to the corresponding acid.

An unusual copper catalysed cyanation of terminal alkyne **2.51**, using AIBN as the nitrile source, was employed to synthesise the propargylic nitrile **2.78** in a 29% yield (Scheme 2.36).[131] Despite extensive optimisation studies, large amounts of starting material **2.51** were recovered from the reaction along with the product. The IR

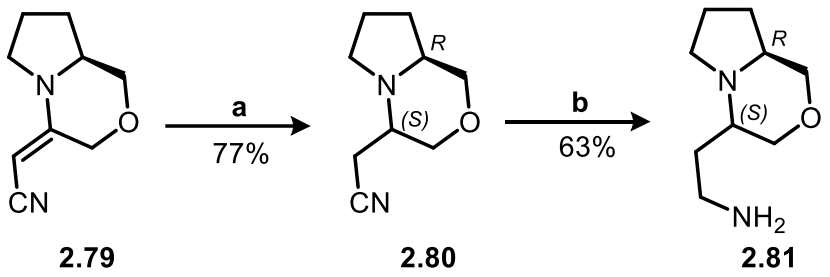


Scheme 2.36: alkyne cyanation and Boc-deprotection/cyclisation. *Reagents and Conditions* **a-** $Cu(NO_3)_2.3H_2O$ (0.2 eq.), AIBN (1.5 eq.), MeCN, 90°C, o/n **b-** TFA, DCM, 0°C.

spectrum of the propargylic nitrile **2.78** exhibited a distinguishing nitrile stretch at 2241 cm^{-1} which along with the loss of the terminal alkyne proton confirmed the structure of **2.78**. Boc-deprotection using TFA in DCM followed by pH adjustment to 9-10 using 2M NaOH yielded the *β*-enamino nitrile **2.79** in a 74% yield.

With the *β*-enamino nitrile **2.79** in hand we were aware that global reduction of the enamine double bond and the nitrile could likely be achieved using a strong reducing agent, such as lithium aluminium hydride. However, to allow for increased flexibility of the synthetic pathway we opted to attempt chemoselective reduction of the enamine, meaning that the nitrile could be transformed to the *C* or *N*-terminus when or if required.

The chemoselective reduction of the enamine double bond of **2.79** was successfully carried out by use of sodium cyanoborohydride and a catalytic amount of HCl to afford the tertiary amine species **2.80** in a 77% yield (Scheme 2.37). The reduction proceeded with complete diastereoselectivity which, due to lack of conclusive nOe or crystal structure could not be definitively assigned. By analogy to the NMR spectra collected after the reduction of the corresponding *β*-enamino ester, we postulated that this reduction had also given the *S,R* diastereoisomer.



2.79 **2.80** **2.81**

Scheme 2.37: *β*-enamino nitrile reduction. *Reagents and Conditions* **a-** $NaBH_3CN$ (1.1 eq.), HCl (cat.), EtOH, 1.5h **b-** $LiAlH_4$ (1.0 eq.), THF, 0°C, 1.5h.

Nitrile **2.80** was then treated with lithium aluminium hydride in THF, which reduced the nitrile to the bicyclic morpholine **2.81** bearing the *N*-terminal chain in a 63% yield. A sharp amine stretch at 3285 cm^{-1} in the IR spectrum was observed.

Amide coupling to the bicyclic morpholine **2.81** to amino acids would cause a reversal in the peptide chain direction in relation to that carried out in section 2.4.8.. This combination with various other modifications demonstrated in this work, and broadens the synthetic flexibility of the morpholine template significantly.

2.5. *β*-Turn Mimetic Biophysical Testing

Following the successful synthesis of a variety of functionalised bicyclic morpholines, discussed throughout this chapter, a series of compounds was sent to LifeArc for biological evaluation. This series of compounds was tested by SPR, the theory of which is described in more detail in Section 2.2.4.. The SPR testing, and sensorgrams displayed in this section were carried out at LifeArc, Stevenage, by Dr Lisa Hale and Joel Burke.

2.5.1. SPR Testing Method

SPR testing was all carried out on a BIAcore T200 machine. The activating protein was immobilised onto a streptavidin SPR sensorchip by biotinylation. Before any tests were carried out the sensorchip was washed with the buffer solution which was to be used throughout the assay to establish an initial baseline. The tested compounds, or analytes were then flowed over the sensorchip at increasing concentrations, with a series of washes followed by a buffer run blank between each analyte to regenerate the immobilised protein. Individual results in each buffer solution are described in the next sections.

2.5.2. 5% DMSO Buffer Solution Tests

A buffer solution containing 5% DMSO in a HBS-P+ solution was employed for initial testing. This buffer system was chosen as not all compounds within the set were suitably soluble in an aqueous only buffer at the required concentrations. The HBS-P+ running buffer solution used, purchased from GE Lifesciences, contained 0.1 M HEPES (a buffering agent), 1.5 M NaCl and 0.5% v/v surfactant, which gave a solution pH of 7.4 when diluted 10x. This running buffer solution on its own had previously been shown to maintain protein stability during testing for the cell surface protein being tested.

A positive control was carried out before any compounds were tested. In which a compound with a known affinity for the activating ligand was flowed over the immobilised protein at various concentrations. The sensorgram, shown in Figure 2.14, shows the response to this positive control. Some super-stoichiometry was observed between the protein and analyte. A slow association and dissociation of binding was seen, presenting as sloped incline and decline of the curve. This interaction is expected for this analyte being ran in a 5% DMSO buffer solution.

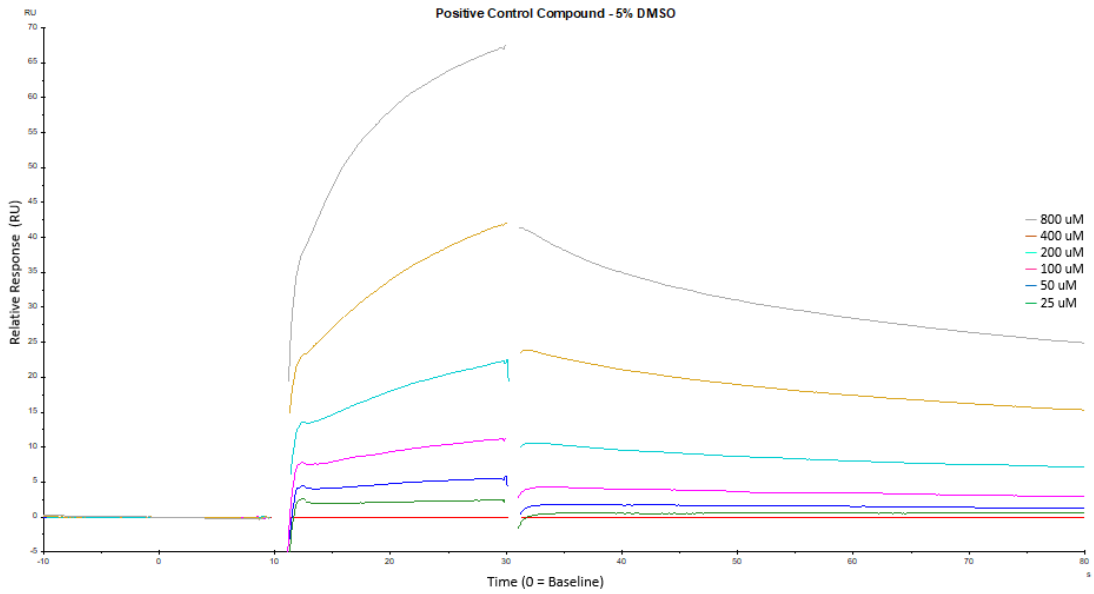


Figure 2.14: Positive control sensorgram running 5% DMSO buffer solution and analyte with known interaction over immobilised protein

The positive control compound was then washed off to regenerate the immobilised protein and a buffer blank wash was conducted. Individual compounds were then ran through as analytes at the same concentration levels. Results of the 5% DMSO buffer solution tests are discussed individually below.

2.5.3. SPR Sensorgram Results of 5% DMSO Buffer Tests

Weak binding of compound **2.56.6** with the immobilised activating ligand, shown in the sensorgram in Figure 2.15. A maximum of 10 RU was achieved at the highest analyte concentration of 800 uM. The R_{max} of the test also seemed suspicious due to the response curve of the results dropping below the established baseline.

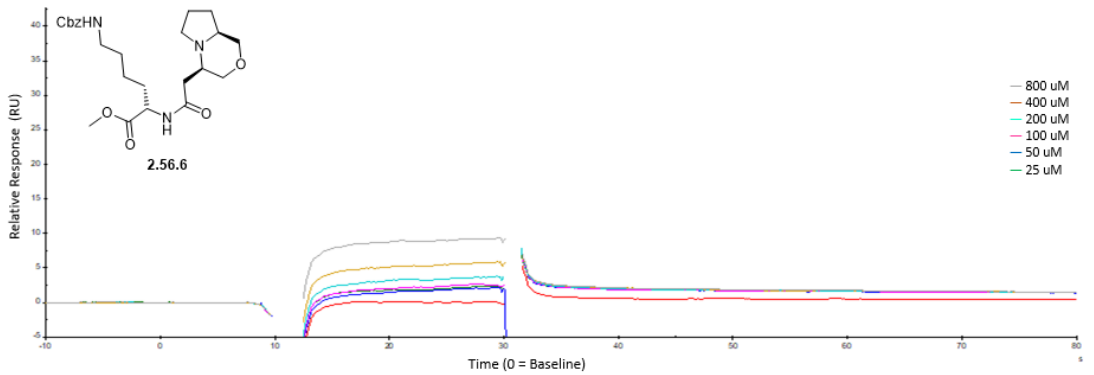


Figure 2.15: Sensorgram of compound **2.56.6** SPR tests at varying concentrations, shown in key, in 5% DMSO buffer solution.

A stronger binding response was observed with compound **2.58**, shown in Figure 2.16. The maximum response more than doubled for the highest concentration of analyte with a 25 RU reading. However, binding of the analyte was inconclusive due to the slow association and dissociation times, indicated by sloping up and down of the graph. This association/dissociation of indicative of non-specific binding of the analyte to the immobilised protein or it could be an effect of precipitation from the buffer solution. Further tests would be required to fully understand the intricacies of this binding, such as ligand/protein co-crystal NMR. It was decided that as the response was relatively low further tests were not warranted.

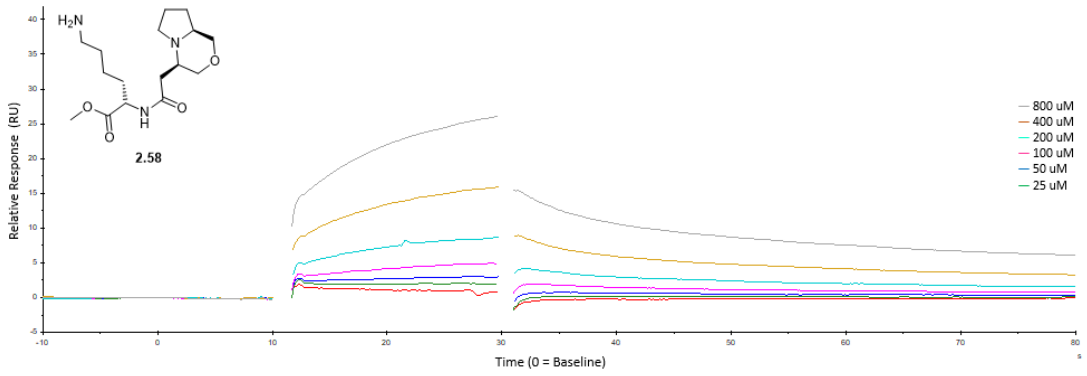


Figure 2.16: Sensorgram of compound **2.58** SPR tests at varying concentrations, shown in key, in 5% DMSO buffer solution.

No other analyte showed any binding to the immobilised protein. In these tests both of the analytes which created responses which may have been affected by the presence of DMSO in the buffer solution. Also, a degree of protein degradation was observed during the running of the set of compounds. This was shown by a second positive control showing a lowering of the maximum response at the same concentrations as previously ran. This degradation could be caused by a number of factors. One of which is the analytes themselves damaging the protein, leaving it unable to bind in subsequent tests. DMSO has also been shown to lead to degradation of proteins during assays when above certain concentrations.[132] The 5% DMSO concentration in theory should not be high enough to cause this, but over a prolonged period of time cannot be discounted as the reason for degradation.

2.5.4. Aqueous Buffer Solution Tests

Having had limited success with the 5% DMSO buffer tests it was decided to conduct further SPR tests using the HBS-P+ buffer solution, herein termed aqueous buffer solution, with no added DMSO. Aqueous soluble compounds only were tested in this process. It was hoped that any unusual activity of the protein analyte interaction would be removed by eliminating all DMSO from the buffer solution, leaving only real interactions to observe.

Once again a positive control was performed before any individual analyte tests were ran. The sensorgram of the positive control test is shown in Figure 2.17. In the aqueous buffer a fast association/dissociation is observed, compared to the slow on/off seen in the same test with the 5% DMSO buffer solution. This fast on/off behaviour in this method of compound screening is desirable. Some non-specific binding is observed at the highest concentrations of 400 and 800 uM, shown by the slight sloping of the sensorgram readouts. The same 6 concentrations of analyte were tested as in previous tests, shown in the key to the right of all sensorgrams.

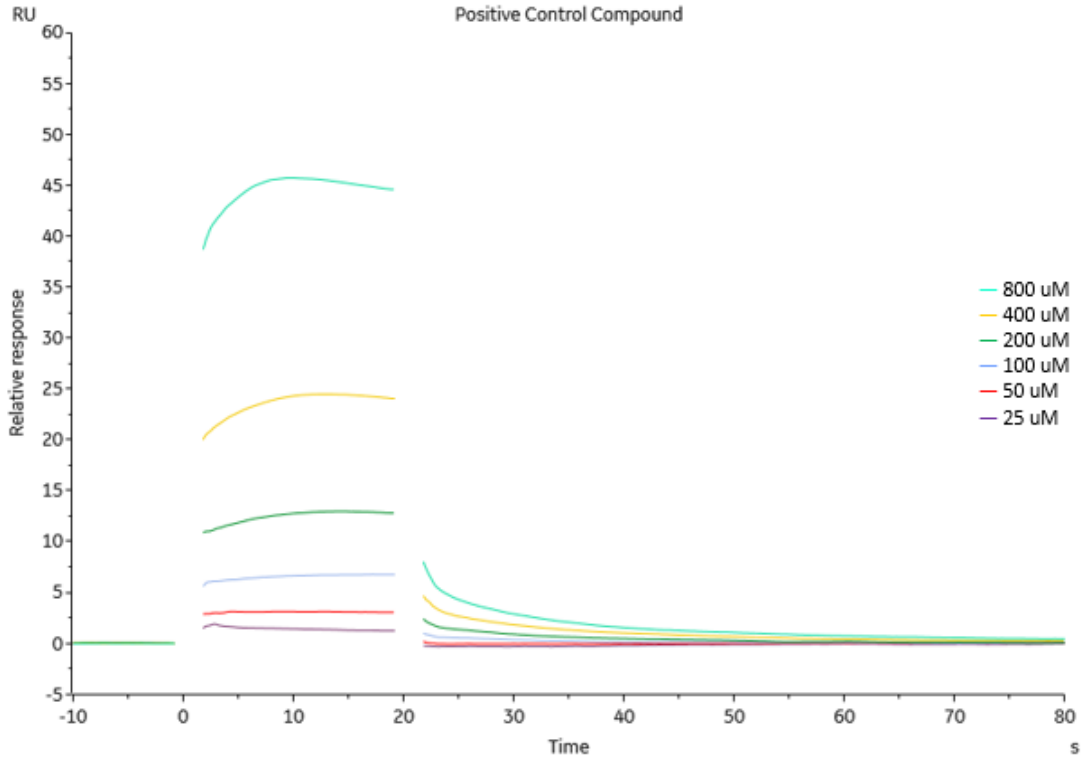


Figure 2.17: Positive control sensorgram running aqueous buffer solution and analyte with known interaction over immobilised protein

Following positive control experiments individual analyte SPR tests were carried out. Individual sensorgrams are discussed in the next section. Only analytes exhibiting a response are discussed, any non-binding interactions were discounted.

2.5.5. SPR Sensorgram Results of Aqueous Buffer Tests

Compound **2.53.1** showed what appeared to be some weak non-specific binding to the immobilised protein (Figure 2.18). A linearity was seen in the association curves, characteristic of non-specific binding. Determination of the specific binding site would require further biophysical techniques, which were not employed because of the weak response shown.

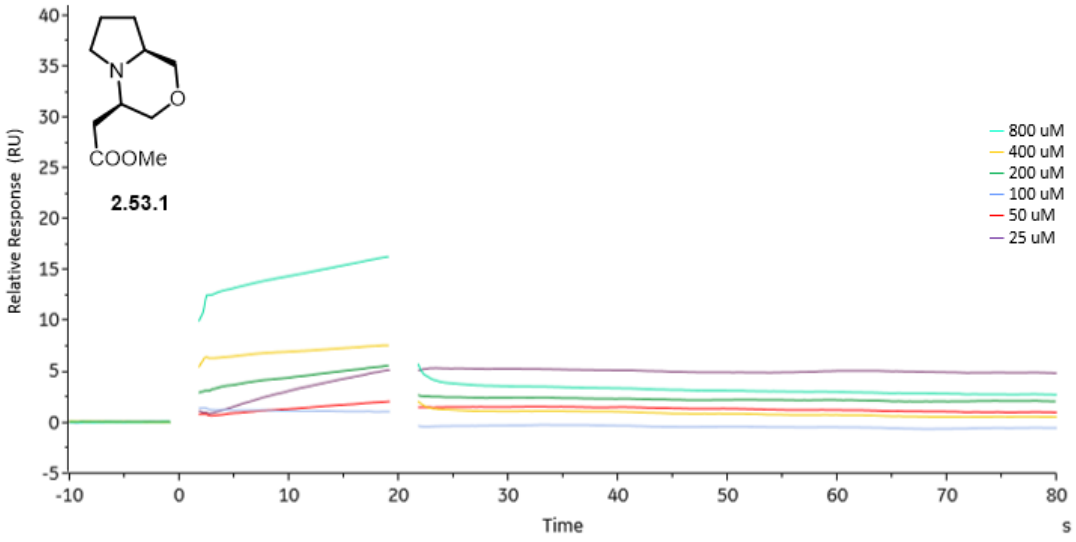


Figure 2.18: Sensorgram of compound **2.53.1** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

The sensorgram for compound **2.57.1** is shown in Figure 2.19. This analyte showed poor compound behaviour in the SPR testing. A slow association and dissociation was observed with the immobilised protein. Compound precipitation was also observed in the aqueous buffer, which can alter refractive index readings. Nevertheless, a weak binding interaction may be being observed. This could not be confirmed without further testing data.

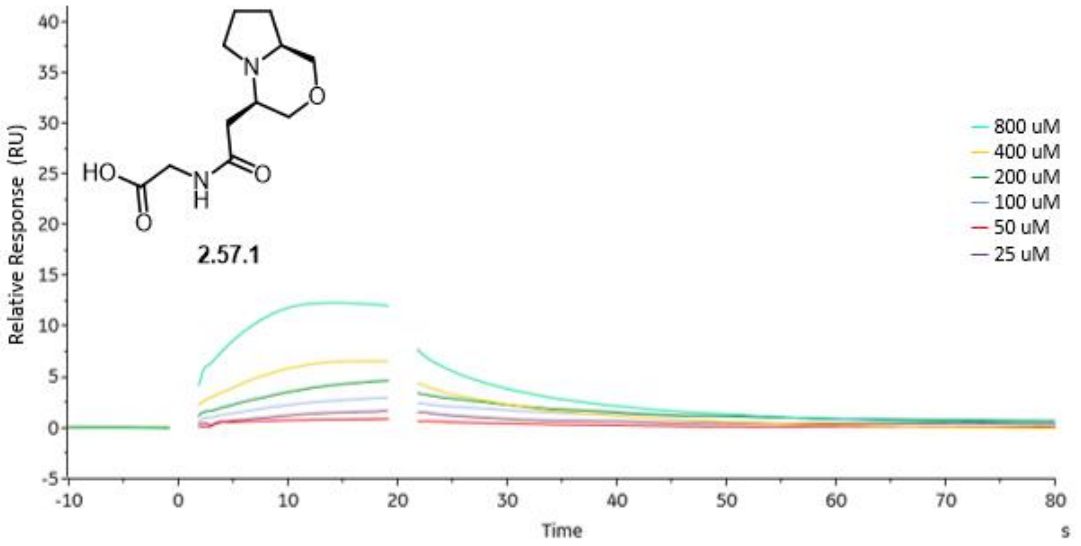


Figure 2.19: Sensorgram of compound **2.57.1** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

Compound **2.56.2** also showed poor compound behaviour. Slow on/off character was yet again observed by the sensorgram readout (Figure 2.20). The possible weak binding was not substantial enough to warrant any further testing. Some non-specific binding is also implied by the shape of the sensorgram.

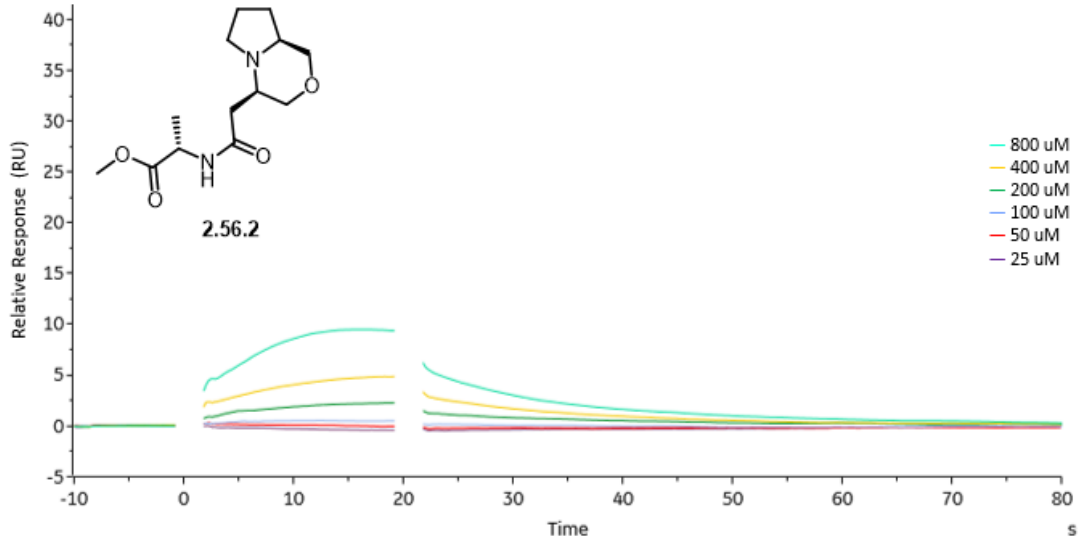


Figure 2.20: Sensorgram of compound **2.56.2** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

The sensorgram for compound **2.56.6**, shown in Figure 2.21, displayed the same poor activity as previously seen in compound **2.56.2**. Compound precipitation during the testing was observed and slow association and dissociation as seen. Due to the poor results shown for this compound no further testing was carried out.

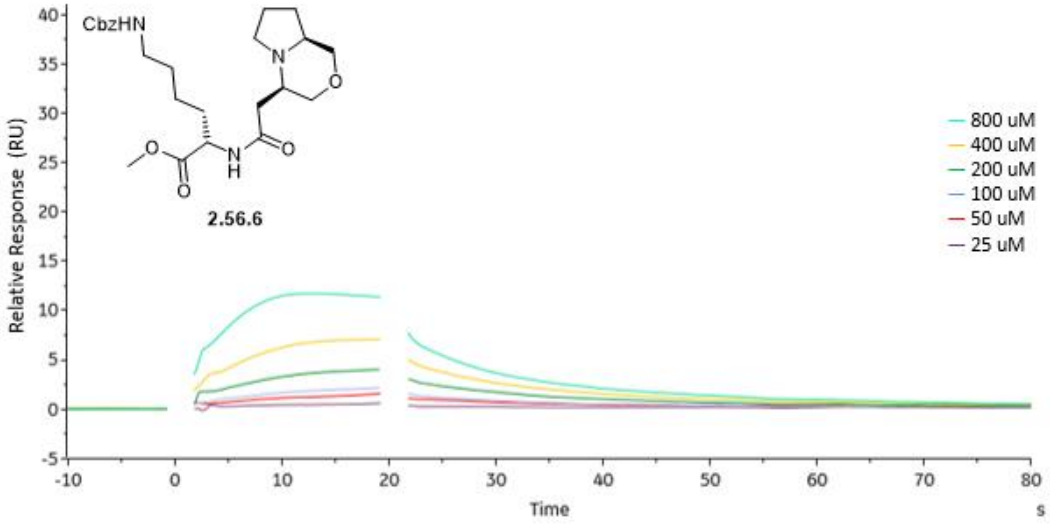


Figure 2.21: Sensorgram of compound **2.56.6** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

Compound **2.56.1** showed a slightly different binding interaction as previously seen in other compounds (Figure 2.22). This is due to a stronger binding interaction was observed in the 400 uM than the more concentrated 800 uM test. This was likely to be an outlier result as discounting this result the association/dissociation is similar to previously tested compounds. Compound precipitation was also observed during the buffer run.

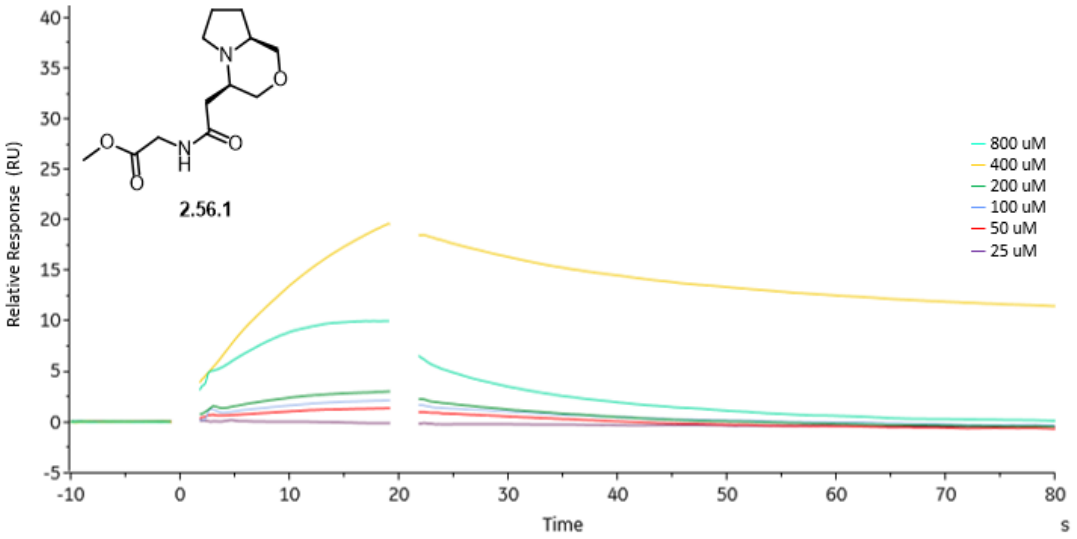


Figure 2.22: Sensorgram of compound **2.56.1** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

Of all of the compounds tested the strongest response was observed when compound **2.56.3** was tested. At a concentration of 800 uM a maximum response of 25 RU was seen. Although this was stronger than that of the other analytes tested it was still relatively low compared to the desired response of a compound of this type. Slow association and dissociation was seen in the same way that it was for all of the analyte which exhibited any binding interactions. Non-specific binding could be the cause of the slow on/off behaviour

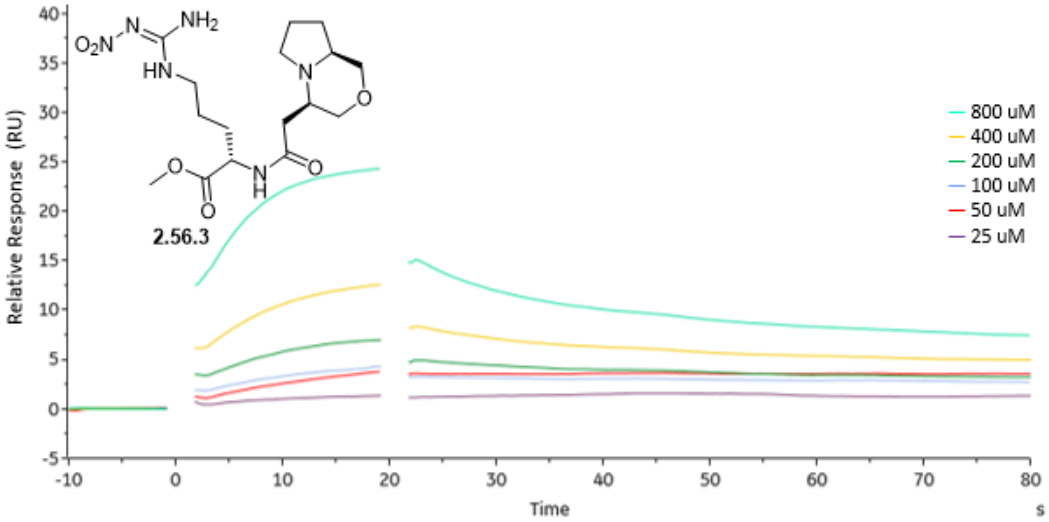


Figure 2.23: Sensorgram of compound **2.56.3** at varying concentrations, shown in key. Tests ran in aqueous buffer solution over immobilised activating protein.

2.5.6. Summary of SPR Biophysical Testing

In summary, 7 of the β-turn mimetic compounds tested elicited low to moderate responses during SPR testing. A 5% DMSO buffer, whilst preferential for compound solubility resulted in a degree of protein degradation throughout the testing process. This meant that results obtained later may be inaccurate as theoretical and experimental R_{max} would drop proportionally to the amount of degradation. An aqueous buffer was used instead for later test to circumvent the degradation caused by the DMSO, which produced in increased number of responses compared to that of the 5% DMSO buffer.

The compounds which provoked a response in the SPR testing on the whole did not behave well as analytes in many of the tests. A substantial number of the described responses showed slow association and dissociation times, this was compounded by precipitation of analyte and possible non-specific binding in some examples.

The results obtained from the SPR testing method does not specify the region at which any interaction is taking place, therefore further biophysical testing would be required to fully understand the binding interaction of these analytes and the immobilised protein. Protein-ligand co-crystal NMR would be the most accurate, as well as difficult practically, way to fully establish the exact interface between the analyte and protein in this interaction.

2.6. Conclusions and Future Work

A very concise, stereocontrolled method of proline derived, functionalised enamine *N*-oxides has been developed, utilising a novel intramolecular tandem Cope elimination/reverse-Cope cyclisation protocol. This method was employed to introduce a variety of functionality onto the morpholine ring of the bicyclic system including, a diverse range of aryl components.

An alternative synthetic route into the bicyclic morpholine ring system was developed *via* a β-enamino ester, made by employing a Boc-deprotection induced intramolecular cyclisation. A variety of derivatives of the bicyclic morpholine system were also synthesised, which offered multiple points of possible functionalisation for later diversification reaction.

Derivatives of the proline derived, bicyclic morpholine system were tested as β-turn mimetics in an active industrial PPI project in conjunction with our industrial sponsors LifeArc, showing the application of these bicyclic system.

Work is ongoing into development of a wider variety of enamine *N*-oxides, a functional group of which there is very little known about in the literature. Applications of such compounds will be investigated, such as measuring their ability to bind to metals. This may lead to the development of novel ligands for catalysis.

Chapter 3

The Enantioselective Synthesis of Functionalised Pyrrolidine *N*-Oxides

3. Chapter 3 – The Enantioselective Synthesis of Functionalised Pyrrolidine *N*-Oxides

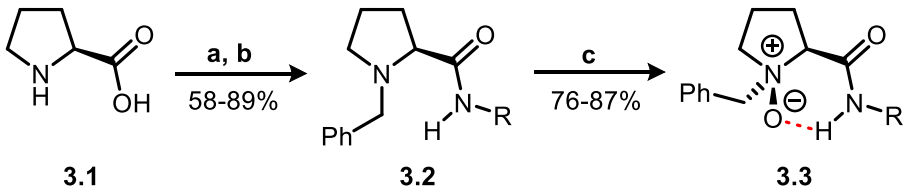
This chapter describes the synthesis of homochiral, pyrrolidine derived tertiary amine *N*-oxides. The aim of this work was to investigate the effect the side chain functionality had on the diastereoselective oxidation of proline derived compounds. The possible reasons behind the observed selectivity will also be discussed.

3.1. Introduction to Enantioselective *N*-Oxide Synthesis in the O'Neil Group

Stereoselectivity is a widely explored area of organic chemistry and an understanding of such reactions is key to developing effective synthetic routes. The direct oxidation of tertiary amines is discussed in Section 1.1.2., the constituent *N*-oxide products of these reactions often exhibit optical activity, and control of this oxidation process is of great interest. The O'Neil group have undertaken extensive work in the stereoselective oxidation of these substrates, and this is the focus of the work described herein.

3.1.1. Hydrogen Bond Directed *N*-Oxidation of *N*-Benzyl Proline Derivatives

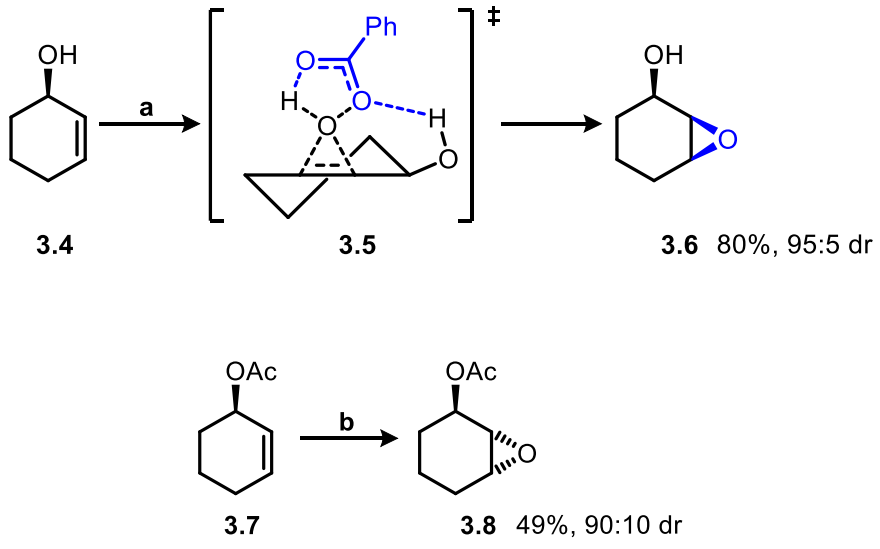
The observed diastereoselectivity in the oxidation of proline derived secondary amides (Scheme 3.1) highlighted the scope of this reaction given the potential of such compounds.[133] A range of amides **3.2** were synthesised by *N*-benzylation and subsequent amide coupling of proline **3.1,** with a variety of alkyl and aryl amines. *N*-Oxidation of the resultant tertiary amines with *m*-CPBA afforded the *N*-benzyl proline derived *N*-oxides **3.3** as single diastereoisomers.



Scheme 3.1: Diastereoselective oxidation of secondary amides. *Reagents and Conditions* **a-** BnBr, *n*-Bu_4NOH, NaOH, H_2O, reflux **b-** RNH_2, DCC/HOBt, DMAP, THF **c-** *m*-CPBA, K_2CO_3, DCM.

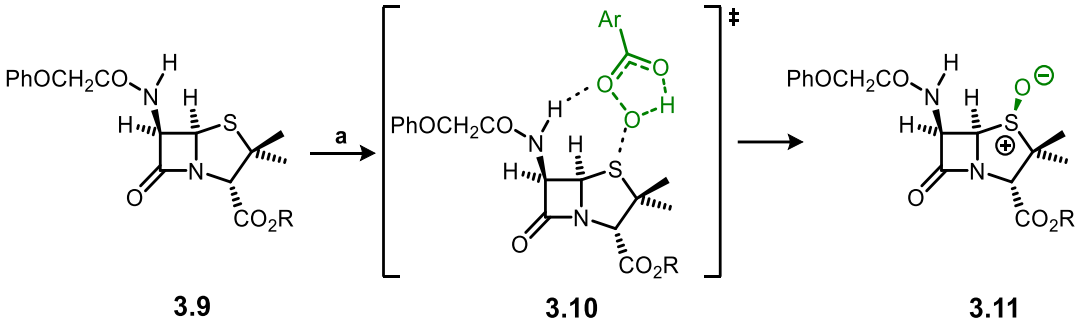
X-Ray analysis of *N*-oxide products showed that the oxidation had proceeded with complete *syn* selectivity relative to the pyrrolidine side chain. Furthermore, an intramolecular H-bond was observed between the amide NH and *N*-oxide oxygen, which stabilized the structure, which existed in its non-hydrated form. It was proposed that the presence of the H-bond donor also directed the oxidation *via* an intermolecular hydrogen bond between the amide and the incoming peracid.

The use of hydrogen bond donors to introduce stereoselectivity in peroxyacid oxidation reactions has been reported in the literature for decades. A 1958 publication by Henbest and Wilson demonstrated that the presence of a suitably positioned hydroxyl group could direct peracid olefin oxidation.[134] The oxidation of 2-cyclohexen-1-ol **3.4** with perbenzoic acid in benzene afforded the *syn*-epoxide **3.6** (Scheme 3.2), whereas oxidation of the corresponding *O*-acetyl protected olefin **3.7** gave the *anti*-epoxide **3.8** preferentially. Mechanistic studies concluded that oxidation of **3.4** was proceeding *via* the transition state **3.5**, where an intermolecular hydrogen bond between the hydroxyl group and incoming peracid directed the epoxidation.[135, 136] Conversely blocking this hydrogen bonding interaction by use of the acetyl protecting group, meant steric interactions led to the *anti*-epoxide **3.8**.



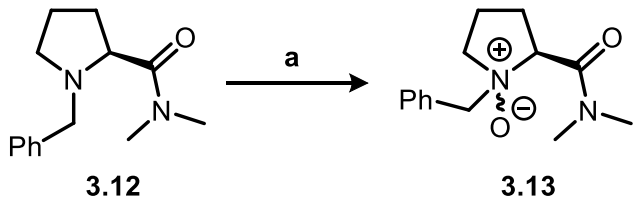
Scheme 3.2: Oxidation of 2-cyclohexene-1-ol and *O*-acetyl-2-cyclohexene-1-ol. *Reagents and Conditions* **a-** $PhCO_3H$, C_6H_6, 2.5 h **b-** $PhCO_3H$, C_6H_6, 31 h.

In 1969 Cooper and co-workers showed that sulfoxidation of a phenoxymethyl penicillin **3.9** with *m*-CPBA proceeded with complete facial selectivity (Scheme 3.3).[137] Interestingly the sulfoxide formed exclusively to the more hindered top face of the penicillin thiazolidine ring, it was postulated that this was as a result of intermolecular hydrogen bonding of the amide NH and the incoming *m*-CPBA molecule in the transition state **3.10** causing oxidation on the more hindered face, yielding **3.11**.



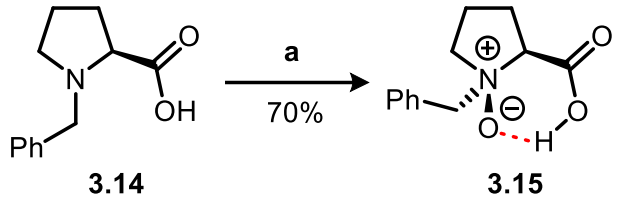
Scheme 3.3: H-Bond driven stereoselective *S*-oxidation of penicillin derivatives using *m*-CPBA.

These examples strongly suggest that the presence of hydrogen bonding is significant in the selective oxidation of proline derived tertiary amines, a theory that was further explored by O'Neil and co-workers in 1993. In this work tertiary amide **3.12** was oxidised in a similar manner as the secondary amide analogues. With no H-bond donor present the oxidation resulted in a mixture of diastereoisomers **3.13** (Scheme 3.4), seemingly confirming the initial hypothesis. The *N*-oxide product also proved unstable when stored at room temperature, presumably due to the lack of the stabilising intramolecular H-bond.



Scheme 3.4: Oxidation of tertiary amide. *Reagents and Conditions* **a-** *m*-CPBA, K_2CO_3, DCM.

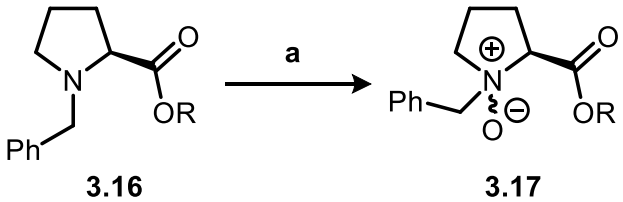
These interesting observations led the group to investigate the scope of this hydrogen bond mediated stereoselectivity in a wider range of proline derivatives. *N*-Oxidation of *N*-benzyl proline **3.14** once again resulted exclusivity in the *syn* diastereomer **3.15** as the only product (Scheme 3.5), unsurprisingly a hydrogen bond between the carboxylic acid and the amine oxide was also observed.[138] Interestingly the oxidation product in this case was actually less polar on TLC that the parent amine starting material; this was thought to be as a result of the aforementioned intramolecular interactions. Moreover X-Ray crystallography determined that the carboxylic acid proton was closer to the amine oxide oxygen than that of the carboxylate, indicating a strong intramolecular interaction.



Scheme 3.5: Oxidation of *N*-benzyl proline. *Reagents and Conditions* **a-** *m*-CPBA, K_2CO_3, DCM.

The same *syn* selectivity was observed for *N*-oxidation of *N*-benzyl prolinol and *N*-benzyl diphenyl prolinol. The crystal structures of these *N*-oxide species displayed an intermolecular, rather than intramolecular hydrogen bond existing between a hydroxyl groups of one molecule to the *N*-oxide of another.

The removal of selectivity in the absence of a hydrogen bond donor in the side chain of the substrate was once again proven in the oxidation of a number of *N*-benzyl proline ester derivatives **3.16** (Scheme 3.6). The tertiary amine *N*-oxide products **3.17** were obtained as a mixture of diastereoisomers (Table 3.1), and were also unstable when stored for prolonged periods of time.



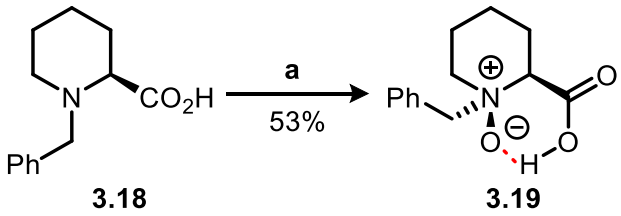
Scheme 3.6: Oxidation of *N*-benzyl proline esters. *Reagents and Conditions* **a-** *m*-CPBA, K_2CO_3, DCM.

Entry	R	3.17 Yield (%)	Ratio *syn* : *anti*
1	Me	85	2 : 1
2	Et	82	4: 1
3	*t*-Bu	88	9: 1

Table 3.1: Oxidation of *N*-benzyl proline esters.

3.1.2. Ring Size Effects on Oxidation Selectivity

These promising results led the group to consider if the same stereocontrol would be observed in substrates with varied ring size. Indeed, *N*-oxidation of *N*-benzyl pipecolic acid **3.18** gave the *N*-benzyl pipecolic acid *N*-oxide **3.19** as a single diastereoisomer (Scheme 3.7), thus suggesting that this process could yield a huge number of potentially exciting and interesting compounds.[139]



Scheme 3.7: *N*-Oxidation of *N*-benzyl pipecolic acid. *Reagents and Conditions* **a**- *m*-CPBA, K_2CO_3, DCM.

X-Ray crystallography showed that the *N*-oxide was formed *cis* to the H-bond donor containing side chain, in line with previous results, with an observable intramolecular hydrogen bond between the acid and amine oxide oxygen. Furthermore, the necessity of the H-bond donor was tested by *N*-oxidation of *N*-benzyl pipecolic esters, as had been done for the 5-membered ring analogues. Again, a mixture of diastereoisomers as was observed, but with slightly larger preference for the *syn* diastereomer than the pyrrolidine counterparts and with somewhat increased stability. These comopounds were also slightly more stable than their proline analogues.

Hydroxyl **3.20** and amide **3.21** containing side chain derivatives also yielded exclusively the *cis* diastereoisomers upon oxidation, again possessing intramolecular hydrogen bonds as all previous examples had as well.[138]

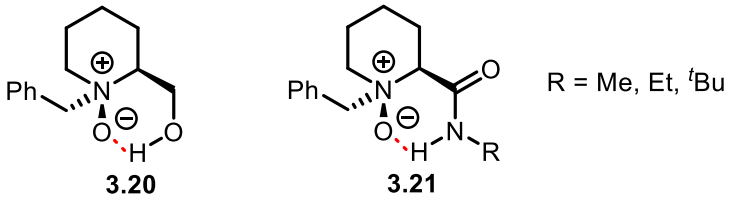
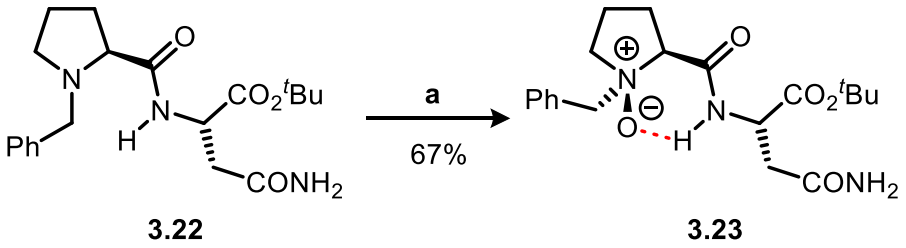


Figure 3.1: *N*-Benzyl pipecolic acid derived *N*-oxides.

3.1.3. Multiple H-Bond Donors

All previously investigated tertiary amines possessed a side chain which contained a lone hydrogen bond donor group, which could form an intramolecular 6-membered ring H-bond interaction with the *N*-oxide oxygen. The effect of *N*-oxidation of systems containing multiple hydrogen bond donors was also explored, with the aim of establishing if any preferential intramolecular bonding patterns would be observed.

Initially, the effects of multiple hydrogen bond donors situated in the side chain of the proline derivatives was explored. In order to achieve this, dipeptide Bn-Pro-Asn-O*t*-Bu **3.22** was synthesised by peptide coupling of *N*-benzyl proline with asparagine *t*-butyl ester (Scheme 3.8).[138] *N*-Oxidation of **3.22** gave *N*-oxide **3.23** as a single diastereoisomer which, in accordance with previous results, had occurred *syn* to the amide side chain.



Scheme 3.8: *N*-Oxidation of Bn-Pro-Asn-O*t*-Bu dipeptide. *Reagents and Conditions* **a-** *m*-CPBA, K_2CO_3, DCM.

Hydrogen bonding was apparent within the structure of **3.23** due to a characteristic shift in the *N*-oxide N-O bond stretch in the IR spectrum. Two conceivable hydrogen bonding interactions were possible within *N*-oxide **3.23**, shown in Figure 3.2, which by NMR analysis was elucidated to occur exclusively between the *N*-oxide and the secondary amide in preference to the more remote primary amide, forming a 6-membered hydrogen bond.

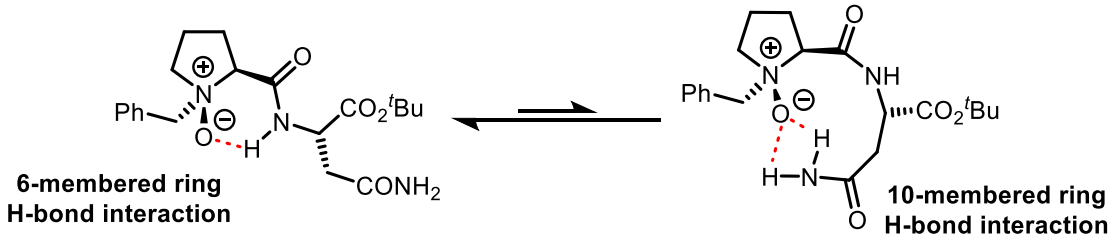
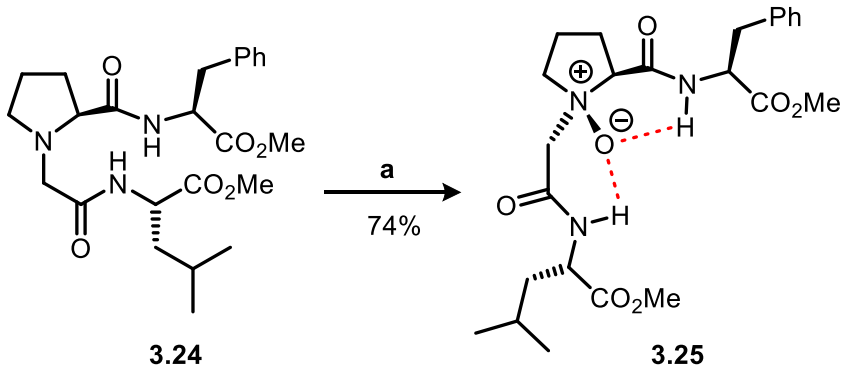


Figure 3.2: Potential hydrogen bonding interactions.

With preference for an intramolecular 6-membered ring hydrogen bonding over more distant interactions established, investigations into multiple simultaneous interactions were carried out. The tripeptide like species **3.24** was synthesised in three steps from Cbz-proline, which offered two amide NHs, both of which could partake in a 6-membered ring intramolecular H-bond (Scheme 3.9). *N*-Oxidation of the tripeptide, as previously seen, proceeded with complete diastereoselectivity, with oxidation occurring *syn* to the phenylalanine side chain to yield **3.25**.



Scheme 3.9: *N*-Oxidation of proline derived tripeptide. *Reagents and Conditions* **a-** *m*-CPBA, K_2CO_3, DCM.

NMR analysis of the *N*-oxide product **3.25** showed significant downfield shifting, approximately 3.5 ppm, of both amide NH peaks, indicating that both amides were in

fact forming hydrogen bonds with the amine oxide oxygen, forming two 6-membered interactions. No X-ray structures of these analogues were obtained therefore the defined stereoselectivity was assigned purely on observations made from the NMR spectra.

3.1.4. Summary of Previous Work

From the results discussed, which were obtained within the O'Neil group in the past, a number of conclusions have been drawn:

- The presence of a hydrogen bond donor, such as OH or NH, in the side chain of pyrrolidine or piperidine rings leads to exclusively *syn*-*N*-oxides upon oxidation of the corresponding tertiary amines.
- Removal of such hydrogen bond donor reduces the *syn*-selectivity of *N*-oxidation to give a mixture of *syn* and *anti* diastereoisomers.
- The presence of an intramolecular hydrogen bond to the *N*-oxide from these side chain functionalities makes the structure considerably more stable.

3.2. Results and Discussion

Following on from the research covered *vide supra*, and with the applications of similar species discussed in Section 1.1.4. in mind, the aim of this section of work was focussed around three main points:

- To explore the scope of functionality which could be incorporated into the proline derived systems, widening the scope of the work already carried out.
- To investigate the limit of H-bonding interaction distance between side chain and amine oxide, in order to observe if loss of *syn* selectivity is lost in the case of more remote H-bond donors.
- To further test the reliance of a H-bond donor presence for oxidation stereoselectivity.

3.2.1. Oxidation of *N*-Benzyl-*L*-Prolinol Carbamates

To date, the scope of functionality introduced into the side chain of the pyrrolidine ring systems has been fairly limited. The vast majority of work, which allows for the stereocontrol of tertiary amine oxidation, has revolved around acid, hydroxyl and amide containing side chains. All of these examples provide 6-membered ring hydrogen bond donors to the amine oxide oxygen, which intramolecularly bind preferentially over any more remote donors present. The scope of diversification is also limited, with the amide side chain derivatives possessing the only opportunity for further chain alterations with no loss of oxidation selectivity.

We proposed the incorporation of a carbamate side chain to the *N*-benzyl proline system (Figure 3.3), which has the NH within the carbamate group as a more remote H-bond donor than exhibited in previous systems. This could hypothetically form an 8-membered ring hydrogen bonding interaction if bonded to the *N*-oxide, as opposed to a 6-membered ring H-bonding previously observed.

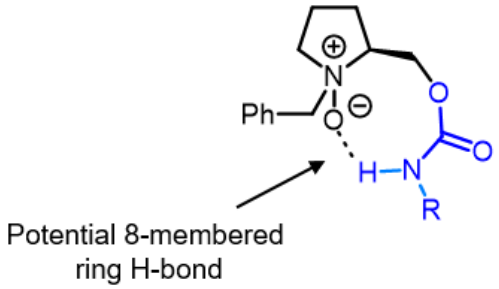
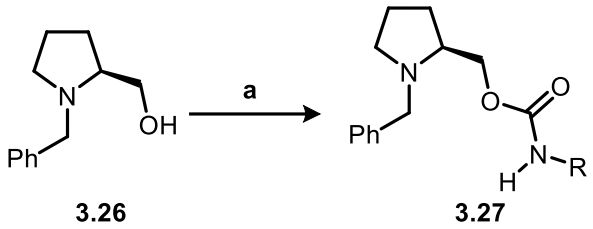


Figure 3.3: Potential intramolecular H-bonding interaction within proposed carbamate derivatives.

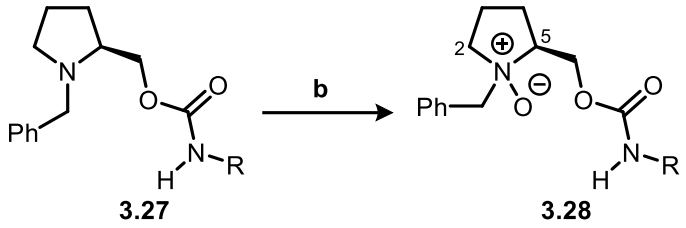
Commercially available *N*-benzyl prolinol **3.26** was deprotonated with sodium hydride to give the alkoxide *in situ*, which was trapped out with a variety of isocyanates (Table 3.2) to give the carbamate derivatives **3.27** in yields ranging from 58-98% (Scheme 3.10). The isocyanates employed were either commercially available or readily synthesised from their corresponding amines.[140] Introduction of the carbamate in this way meant a diverse range of substituent R groups could be attached to the *N*-benzyl prolinol system.



3.26 **3.27**

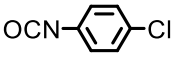
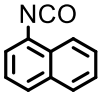
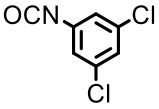
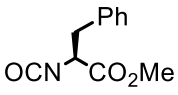
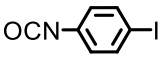
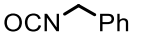
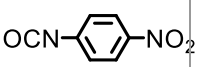
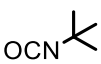
Scheme 3.10: Synthesis of *N*-benzyl prolinol carbamate derivatives. *Reagents and Conditions* **a-** (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) RNCO (1.2 eq.), o/n.

Oxidation of the carbamate **3.27** derivatives was carried out by treatment with *m*-CPBA at -78°C in DCM, with a K_2CO_3 buffer to give the *N*-oxide products **3.28** in good yields ranging from 75-95% (Scheme 3.11).



Scheme 3.11: Synthesis of *N*-benzyl prolinol carbamate *N*-oxide derivatives. *Reagents and Conditions* **a-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

Significant downfield shifting of approx. 1 ppm was seen for the benzylic CH_2 and protons of carbons 2 and 5, indicative of *N*-oxide formation due to the inductive effect of the positively charged nitrogen. The K_2CO_3 buffer was used to neutralise the stoichiometric amounts of *m*-chlorobenzoic acid produced during the oxidation, which could potentially alter the oxidation process, by preventing protonation of unreacted starting material.

Entry	Isocyanate	3.27 Yield (%)	3.28 Yield (%)	Entry	Isocyanate	3.27 Yield (%)	3.28 Yield (%)
1		**77**	**85**	**5**		**59**	**80**
2		**83**	**88**	**6**		**58**	**89**
3		**79**	**75**	**7**		**82**	**95**
4		**82**	**95**	**8**		**98**	**92**

Scheme 3.2: Yields of formation of *N*-benzyl prolinol carbamate derivatives and *N*-oxidation reactions.

Interestingly, all *N*-oxide products **3.28** were obtained as **single diastereomers**. The *N*-oxide products were isolated as single spots by TLC, with no doubling of signals observed in ^{13}C NMR spectra. Reactions were also monitored by ^{1}H NMR to confirm that no minor diastereoisomer was missed during the purification process. The majority of *N*-oxide products were afforded as solid, foam like substances, which were all highly stable compounds that could be stored for prolonged periods of time with no observable decomposition.

An X-ray crystal structure was obtained of the *t*-butyl carbamate *N*-oxide (Figure 3.4), which confirmed that *N*-oxidation had occurred exclusively *syn* to the carbamate side chain. However, in contrast to previous work no intramolecular hydrogen bond was identified within the crystal structure. There was also no water of co-crystallisation to the *N*-oxide which may have been expected in the absence of an intramolecular hydrogen bond. To date the proline derived carbamate derivatives possess the most remote H-bond donor group in the diastereoselective *N*-oxidations of these systems.

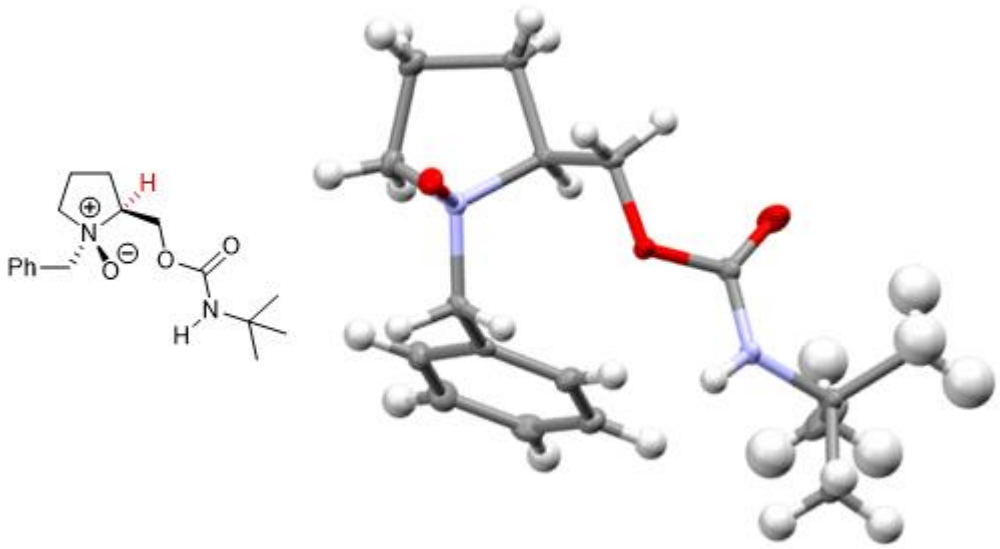
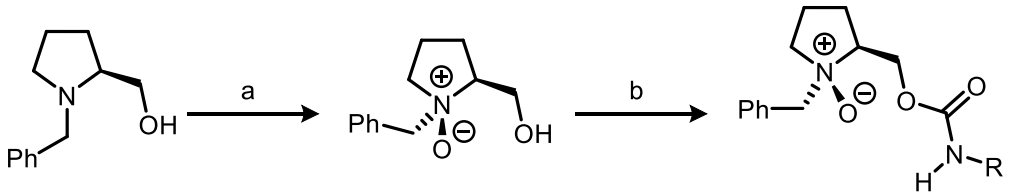


Figure 3.4: X-ray crystal structure of proline derived ^{t}-butyl carbamate *N*-oxide.

All carbamate *N*-oxide derivatives were also synthesised by an unambiguous route to confirm *syn* oxidation selectivity (Scheme 3.12). *N*-Oxidation of *N*-benzyl prolinol **3.26** by treatment with *m*-CPBA afforded the *syn N*-benzyl prolinol *N*-oxide **3.29** in 95% yield as a crystalline white solid.[138] Deprotonation of **3.29** by treatment with sodium hydride, followed by addition of the respective isocyanates yielded the *N*-benzyl proline carbamate derivatives **3.28** in yields ranging 75-95%. Having fixed the geometry of the systems by oxidising prior to carbamate formation the *syn N*-oxide adducts were exclusively obtained from this route. Comparison of NMR data from the two alternative routes showed that identical products had been obtained, further confirming that *syn*-selectivity had been exclusive in the initial route. Using this approach, there is a great potential for the synthesis of a range of chiral proline derivatives



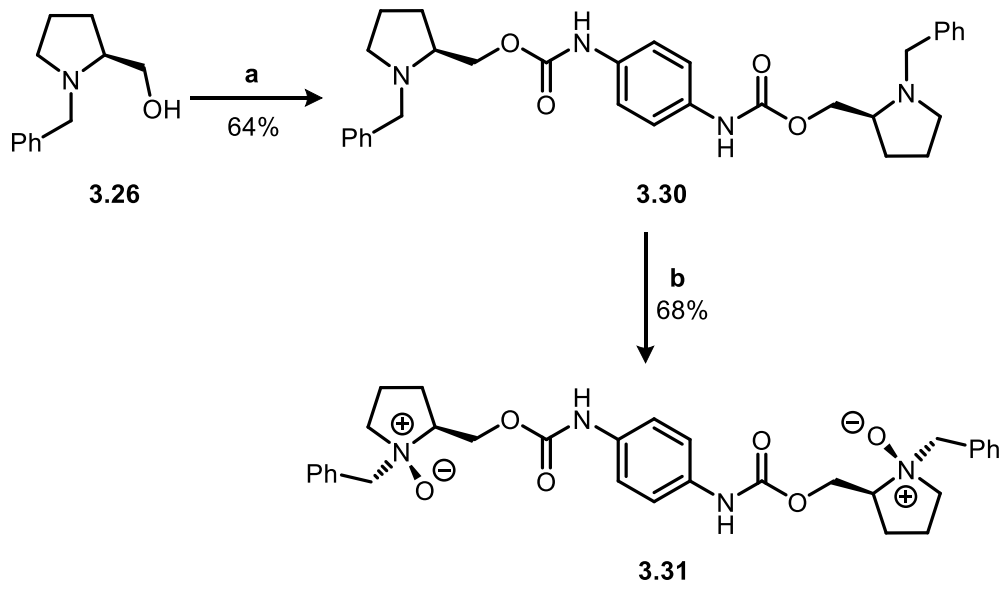
3.26 **3.29** **3.28**

Scheme 3.12: Alternative synthesis of *N*-benzyl prolinol carbamate *N*-oxide derivatives. *Reagents and Conditions* **a-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n. **b-** (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) RNCO (1.2 eq.), o/n.

3.2.2. Synthesis of *N*-Benzyl Proline Derived Carbamate Bis-*N*-Oxides

The Feng group have had great success using C_2-symmetric *N,N'*-dioxides for asymmetric catalysis, as covered in Section 1.1.4., which led us to prepare our own versions of these structures, incorporating this carbamate functionality.[65, 67-69]

We first set about linking the dimer by the pyrrolidine carbamate side chain (Scheme 3.13). Deprotonation of *N*-benzyl prolinol **3.26** with sodium hydride, followed by addition of 0.5 equivalents of 1,4-phenylene diisocyanate gave the carbamate dimer **3.30** in 64% yield. *N*-Oxidation by treatment with a slight excess of *m*-CPBA (2.2 eq.) gave the bis-*N*-oxide **3.31**, as a single diastereoisomer, in a 68% yield.



Scheme 3.13: Pyrrolidine side chain linked carbamate *N*-oxide dimer synthesis. *Reagents and Conditions* **a-** (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) 1,4-phenylene diisocyanate (0.5 eq.), o/n **b-**.*m*-CPBA (2.2 eq.), K_2CO_3 (2.5 eq.) DCM, -78°C, o/n.

A crystal structure was obtained of the *N*-linked carbamate bis-*N*-oxide **3.31**, as shown in Figure 3.5, which confirmed that as expected, the oxidation had occurred *syn* to the carbamate side chain. As with the monomer version there are no intramolecular hydrogen bonds observed within the structure. However, in this case two molecules of water co-crystallised with the compound, H-bonding to the *N*-oxide oxygens. These H_2O molecules have been removed from the image for clarity.

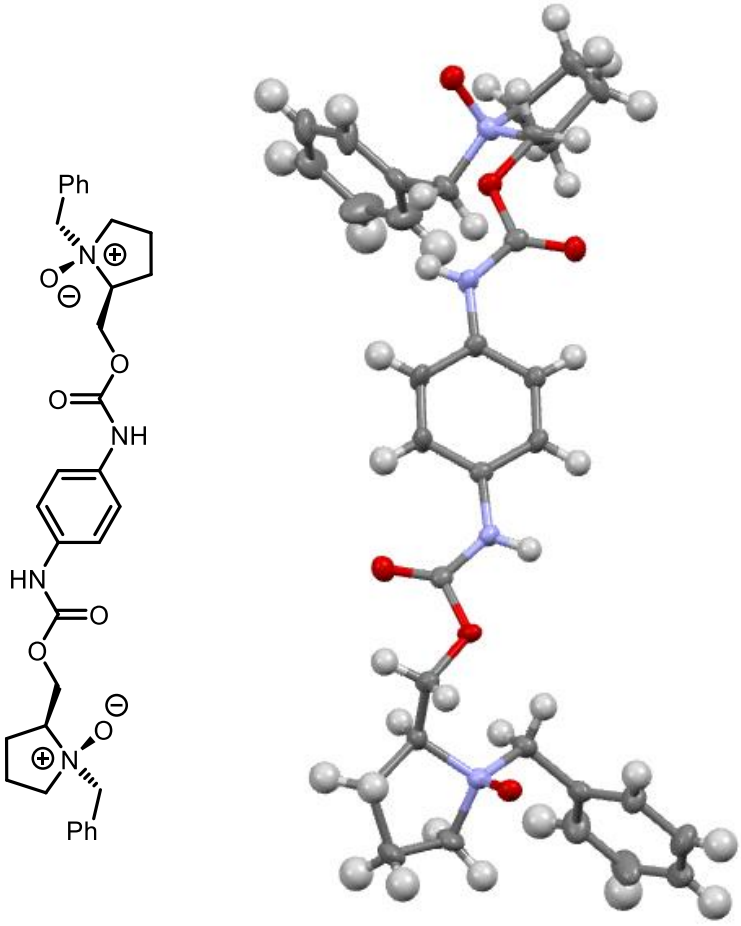


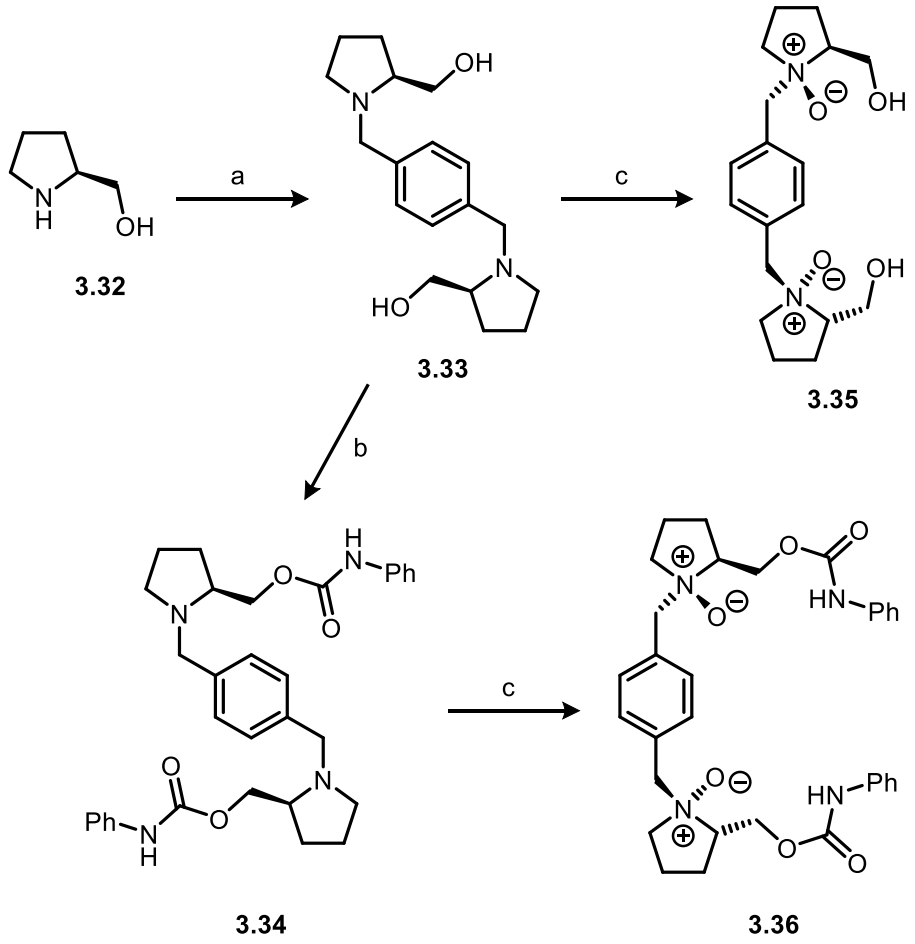
Figure 3.5: Crystal structure of *O*-linked carbamate bis-*N*-oxide **3.31**.

Having successfully synthesised the carbamate side chain linked dimer, we then examined the *N*-linked bis-*N*-oxides (Scheme 3.14). Prolinol **3.32** was heated with *p*-xylene dibromide in toluene for 48 hours to give the xylene linked prolinol dimer **3.33** in 80% yield. Due to the symmetry of the dimer system, signals from opposing sides of the structure coalesced. Alkyl linked pyrrolidines were considered but xylene linked dimers offered a direct comparison to the successful results already obtained.

Oxidation of the parent tertiary amine dimer **3.33** was carried out, using *m*-CPBA, to give the bis-*N*-oxide **3.35** in 64% yield. As expected, due to the similarity of the *N*-benzyl prolinol oxidation, this was obtained as a single diastereoisomer.

The *N*-linked carbamate dimer **3.34** was prepared in 87% yield from the double deprotonation of the diol **3.33** with NaH followed by the addition of phenyl isocyanate.

Subsequent oxidation of both tertiary amines with *m*-CPBA proceeded in 92% yield to give the *N*-linked carbamate bis-*N*-oxide **3.36** as a single diastereoisomer. Downfield shift of the C-α proton from 2.96 ppm in **3.34** to 3.98 ppm in the bis-*N*-oxide **3.36** was observed, indicative of *N*-oxide formation.

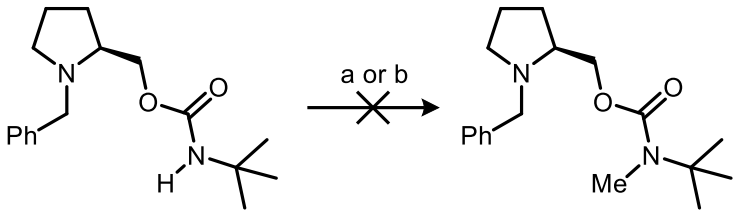


Scheme 3.14: *N*-Linked carbamate *N*-oxide dimer synthesis. *Reagents and Conditions* **a-** *p*-xylylene dibromide (0.5 eq.), K_2CO_3 (1.5 eq.), toluene, reflux, 48h **b-** (i) NaH (2.2 eq.), THF, 0°C, 30 mins (ii) phenyl isocyanate (2.2 eq.), o/n **c-**.*m*-CPBA (2.4 eq.), K_2CO_3 (2.5 eq.) DCM, -78°C, o/n.

3.2.3. Oxidation of *N*-Benzyl-*L*-Proline Derived *N,N*-dialkylcarbamates

We also wished to investigate the significance of the hydrogen bond donor in the carbamate side chain on the *N*-oxidation of these systems. To do this we proposed removing the NH hydrogen by *N*-methylation. The *N*-methylated carbamate could then be oxidised, so that diastereoselectivity could be measured. Numerous attempts

to *N*-methylate the carbamate NH were made (Scheme 3.15). Initially, carbamate **3.27.8** was treated with NaH at 0°C followed by addition of methyl iodide, however no conversion to product **3.37** was observed, and the majority of the starting material was recovered. Subsequent attempts used increased amounts of base and the reaction was heated, but were also unsuccessful. *n*-BuLi was also used instead of NaH but also lead to no product formation.

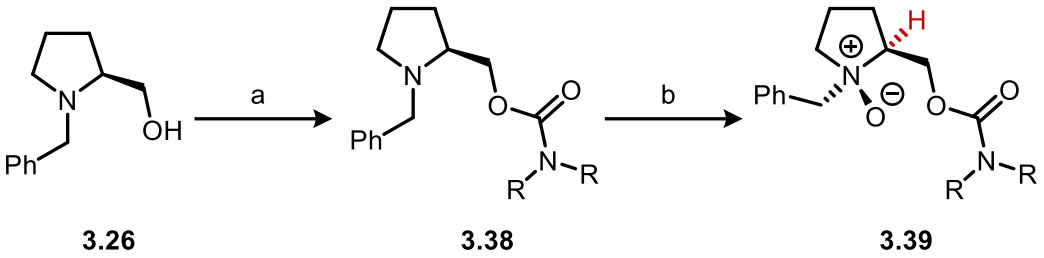


3.27.8 **3.37**

Scheme 3.15: Attempted *N*-methylation of carbamate. *Reagents and Conditions*
a- (i) NaH (1.0-2.5 eq.), DMF, 0°C to reflux, 30 mins (ii) MeI (1.0-2.5 eq.), o/n
b- (i) *n*-BuLi (1.1 eq.), THF, 30 mins, -20°C (ii) MeI (1.5 eq.) 48h.

It is unlikely that unsuccessful deprotonation is the reason for the failure of this reaction. The carbamate pK_a is approximately 21, whereas NaH and *n*-BuLi have pK_as of 35 and 50 respectively. Potentially steric bulk around the carbamate NH could have played a role in the failure of the reaction. Nevertheless, alternative routes of *N*-alkylating the carbamate NH were explored.

The *N*,*N*-dialkylated carbamate derivatives **3.38** were instead synthesised by a carbamoylation reaction of the *N*-benzyl prolinol hydroxyl group (Scheme 3.16).[141] *N*-Benzyl prolinol **3.26** was deprotonated with NaH followed by addition of a dialkyl carbamoyl chloride to give the *N*,*N*-dialkylated carbamate derivatives **3.38**. Subsequent oxidation of **3.38** with *m*-CPBA afforded the *N*,*N*-dialkyl carbamate *N*-oxides **3.39** in good yields (Table 3.3).



Scheme 3.16: Preparation of *N,N*-dialkylcarbamates. *Reagents and Conditions* **a**- (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) R_2NCOCl (1.2 eq.), reflux, o/n. **b**- *m*-CPBA (2.4 eq.), K_2CO_3 (2.5 eq.) DCM, -78°C, o/n.

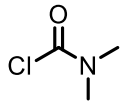
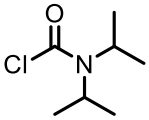
Entry	R_2NCOCl	3.38 % Yield	3.39 % Yield
1		68	84
2		74	95

Table 3.3: Yields of *N,N*-dialkyl carbamate formations and *N*-oxidations

Surprisingly, the *N,N*-dialkyl carbamate *N*-oxides **3.39** both formed as single diastereomers. Upon treatment of the carbamate derivatives with *m*-CPBA crude ^{1}H and ^{13}C spectra showed the presence of single products in both cases. The key C-α proton of **3.39**, marked in red in Scheme 3.16, appears at 3.99 ppm for both analogues. This is virtually identical to all of the previously synthesised carbamate derivatives **3.28**, which all possess the *N*-oxide *syn* to the carbamate side chain. Because of this we suspected that oxidation was still maintaining exclusively *syn* selectivity, with no H-bond donor present in the pyrrolidine side chain. The *N,N*-dialkyl carbamate *N*-oxides **3.39** were both isolated as viscous oils, which were stable under ambient conditions for prolonged periods of time. This is in contrast to previous work, such as the oxidation of ester derivatives **3.16** (Section 3.1.1.), in which a lack of H-bond donor led to products which decomposed relatively quickly.

Due to these unexpected results the dependence of oxidation selectivity on the presence of a H-bond donor was explored in more detail. This will be discussed in Section 3.2.5..

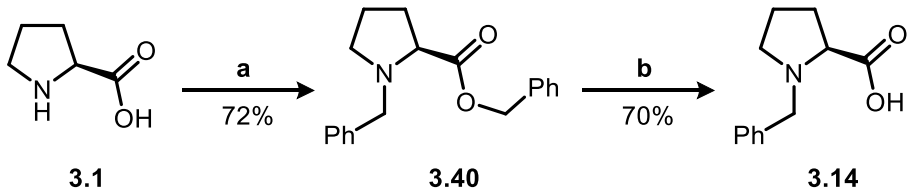
3.2.4. Further Side Chain Functional Group Scope

We wished to further investigate the scope of functional group incorporation into the pyrrolidine side chain of these proline systems. Having the ability to tailor the specific chemical properties of these systems would be very advantageous for any potential future use of these sort of compounds in a wide range of applicaitons.

3.2.4.1. Oxidation of *N*-Benzyl-*L*-Proline Hydroxamic Acids

Incorporation of a hydroxamic acid side chain into the *N*-benzyl pyrrolidine system was of particular interest to us. Hydroxamic acids have been shown to possess very good metal binding properties, particularly to iron.[142-144] Building in a side chain which not only directs *N*-oxidation, but also increases metal binding properties of the overall system, would potentially be very advantageous.

N-Benzyl proline **3.1** was required as the starting material for hydroxamic acid synthesis. To make **3.14**, *L*-proline was first globally benzylated with benzyl bromide in DMF, affording *N*-benzylproline benzyl ester **3.40** in a 72% yield (Scheme 3.17). The benzyl ester was then selectively cleaved by hydrogenation in the presence of palladium on carbon, which gave *N*-benzyl proline **3.14** in a 70% yield. Progress of the hydrogenation was monitored by TLC closely to optimise conversion to product, with minimal *N*-debenzylation taking place.

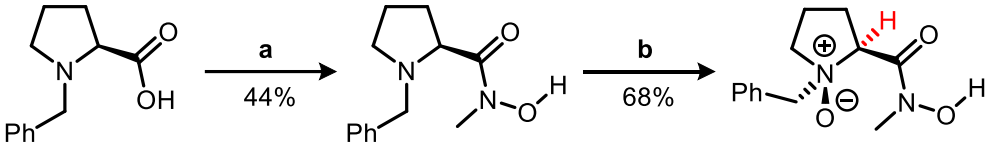


Scheme 3.17: Preparation of *N*-benzyl proline. *Reagents and Conditions* **a-** BnBr, K_2CO_3, DMF **b-** Pd/C (0.1 eq.), H_2, MeOH.

The hydroxamic acid side chain was then made using the *N*-benzyl proline **3.14** *via* the acyl chloride (Scheme 3.18). **3.14** was treated with ethyl chloroformate and triethylamine in THF generating the acyl chloride intermediate *in situ*. Addition of *N*-

methyl hydroxylamine hydrochloride led to nucleophilic acyl substitution to give the hydroxamic acid **3.41** in a 44% yield. HRMS as well as the appearance of a methyl singlet at 3.07 ppm in the ^{1}H NMR confirmed product formation.

With the hydroxamic acid successfully prepared oxidation of the tertiary amine was carried out. Oxidation was achieved under the normal conditions with *m*-CPBA in DCM at -78 ℃ in the presence of K_2CO_3. This afforded the tertiary amine *N*-oxide **3.42** in a 68% yield as a white solid.



3.14 **3.41** **3.42**

Scheme 3.18: Preparation of *N*-methyl hydroxamic acid side chain *N*-oxide. *Reagents and Conditions* **a-** (i) EtO_2CCl (1.2 eq.), NEt_3 (1.2 eq.), THF, 0°C, 1h (ii) NHMeOH.HCl (1.0 eq.), KOH (1.0 eq.), o/n **b**- *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

N-Oxide **3.42** was formed as a single diastereoisomer, as shown by the single sets of peaks seen in both the ^{1}H and ^{13}C NMR spectra. No doubling up or broadening of signals was observed. nOe experiments of **3.42**, irradiating the C-*α* proton (shown in red), showed interactions with the protons of the benzyl CH_2 group (Figure 3.6). This established that oxidation had taken place exclusively *syn* to the hydroxamic acid. This is the first example of a 7-membered H-bond donor in the side chain of these systems being used to control the *N*-oxidation diastereoselectivity. Although not carried out in this work, there is the ability to build in diversity into these hydroxamic acid side chains. As with the carbamate derivatives, the scope is limited only by the availability of the *N*-alkylated hydroxylamines required for product formation.

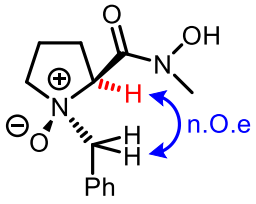
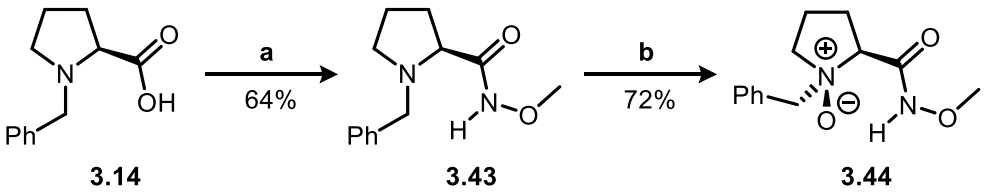


Figure 3.6: nOe interaction between C*α* proton, in red, and benzylic CH_2 proton.

By using *O*-methyl hydroxylamine hydrochloride in the amide coupling reaction the *O*-methyl hydroxamate **3.43** was synthesised from **3.14** in a 64% yield (Scheme 3.19). A clear NH peak at 3192 cm^{-1} in the IR spectrum and a methyl singlet was observed at 3.82 ppm in the ^{1}H NMR confirming product formation.



Scheme 3.19: Preparation of *O*-methyl hydroxamic acid side chain *N*-oxide. *Reagents and Conditions* **a-** (i) EtO_2CCl (1.2 eq.), NEt_3 (1.2 eq.), THF, 0°C, 1h (ii) $NH_2OMe.HCl$ (1.0 eq.), KOH (1.0 eq.), o/n **b-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

Oxidation of hydroxamate **3.43** with *m*-CPBA gave the tertiary amine *N*-oxide **3.44** in 72% yield. As with the *N*-methyl hydroxamic acid analogue the *N*-oxide was formed as a single diastereomer. nOe experiments, shown in Figure 3.7, once again confirmed that oxidation had occurred entirely on the top face of the system. Irradiation of the C-*α* proton (H*) showed interactions with the three protons shown in green, red and blue. Confirmation experiments were also carried out irradiating the benzylic proton (in blue) which showed and interaction with the C-*α* proton as expected. The selectivity of this oxidation was not particularly surprising, as numerous examples of 6-membered H-bond donors controlling *N*-oxidation have been previously shown in the group.

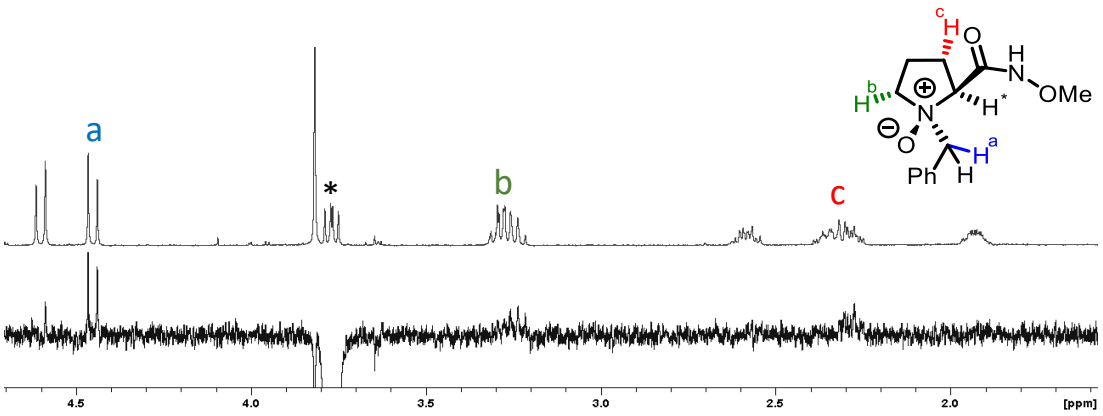
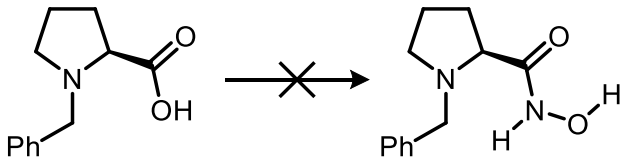


Figure 3.7: nOe of *O*-methyl hydroxamate *N*-oxide.

Although not a surprising result this does allow for an additional point of functionalisation for these systems. A wide variety of *O*-alkyl hydroxylamines are commercially available which can be introduced using the synthetic pathway outlined above. It is worth noting that attempts were made to synthesise the parent hydroxamic acid analogue, shown in Scheme 3.20, by reaction between hydroxylamine hydrochloride and *N*-benzyl proline **3.14** under a variety of amide coupling conditions. No hydroxamic acid **3.45** was isolated from the reactions under any of the attempted conditions, which was attributed to unsuccessful purification of the highly polar product.

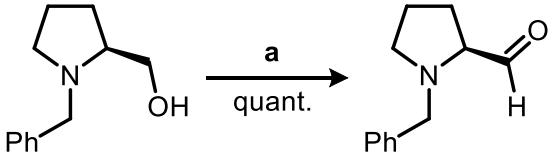


3.14 **3.45**

Scheme 3.20: Attempted parent hydroxamic acid analogue synthesis

3.2.4.2. Oxidation of *N*-Benzyl-*L*-Prolinol Oximes

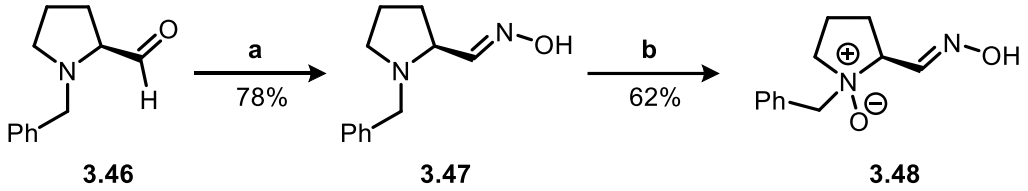
Having shown success with both the carbamate and hydroxamate analogues we wished to diversify the pyrrolidine side chain even further. We attempted to introduce an oxime onto the ring system. To do this we first made *N*-benzyl prolinal **3.46** by Swern oxidation, following a literature procedure (Scheme 3.21).[145] Swern oxidation proceeded in quantitative yield, with no observed racemisation of the carbon centre due to maintenance of low temperatures throughout the reaction process, and the aldehyde product **3.46** was carried forward into the oxime formation reactions.



3.26 **3.46**

Scheme 3.21: Preparation of *N*-benzyl prolinal. *Reagents and Conditions* **a-** DMSO (3.0 eq.), $(COCl)_2$ (1.5 eq.), NEt_3 (4.0 eq.), DCM, -78°C.

A condensation reaction of *N*-benzyl prolinal **3.46** with hydroxylamine hydrochloride afforded the oxime side chain furnished pyrrolidine **3.47** in a 78% yield. The distinctive vinylic carbon peak was observed at 154.0 ppm as well as the broad hydroxyl OH peak at 8.00 ppm in the ^{1}H NMR. Oxidation of the tertiary amine **3.47** gave the related *N*-oxide **3.48** in a 62% yield. The characteristic downfield shift of the C-*α* proton was observed, from 3.12 ppm in **3.47**, to 4.23 ppm in the *N*-oxide **3.48**.



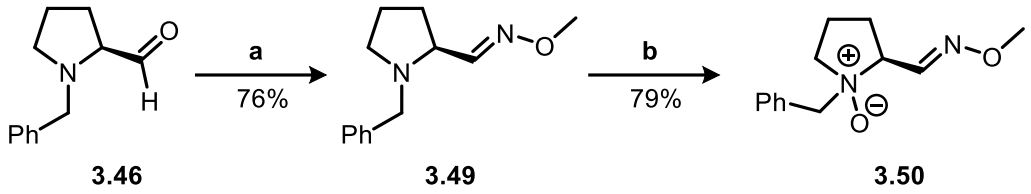
3.46 **3.47** **3.48**

Scheme 3.22: *Reagents and Conditions* **a-** $NH_2OH.HCl$ (2.5 eq.), NEt_3 (2.5 eq.), THF, o/n **b-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

The *N*-oxide species **3.48** was highly stable, being stored for prolonged periods of time with no degradation. As the previous examples discussed, the *N*-oxide **3.48** formed as a single diastereoisomer, however the diastereoselectivity could not be established due to lack of crystal structure, or successful nOe experiments. Also, no suitably analogous *N*-oxides had been previously synthesised, so comparisons could not be made to decipher *syn*/*anti* geometry. We suspected oxidation had occurred *syn* to the pyrrolidine side chain, despite the lack of definitive data. The oxidation of the hydroxamic acid **3.44**, also bearing a 7-membered ring H-bond donor, had proceeded exclusivity with *syn* selectivity. Therefore, we might expect this oxidation process to proceed in the same way.

Although the selectivity of *N*-oxidation had not been determined for the oxime **3.48** we wished to investigate the effect of H-bond donor removal in the side chain. To achieve this, *O*-methyl hydroxylamine hydrochloride was coupled to *N*-benzylprolinal. This gave the *O*-methyl oxime species **3.49**, produced in a 76% yield. As with the parent oxime **3.47**, the *O*-methyl analogue **3.49** was identified by the distinguishing vinylic carbon at 152.7 ppm. Subsequent oxidation with *m*-CPBA gave

the *N*-oxide **3.50** in a 79% yield. C-*α* dowfield shift in the ^{1}H NMR from 3.10 ppm to 4.23 ppm, as well as HRMS, confirmed *N*-oxide formation.



Scheme 3.23: *Reagents and Conditions* **a-** $NH_2OMe.HCl$ (2.5 eq.), NEt_3 (2.5 eq.), THF, o/n **b-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

Oxidation of tertiary amine **3.49** gave *N*-oxide **3.50** as a single diastereoisomer. This result was somewhat unexpected, as previous work removing side chain H-bond donors suggested that there would be a loss of selectivity. Confirmation of oxidation selectivity was not obtained for the same reasons outlined for the parent oxime *N*-oxide **3.50**. However the C-*α* signal in the ^{1}H NMR of both oxime derivatives was identical, at 4.23 ppm. This would suggest that both had formed on the same face of the pyrrolidine ring system, as forming on opposite sides would cause a significant discrepancy between the two.

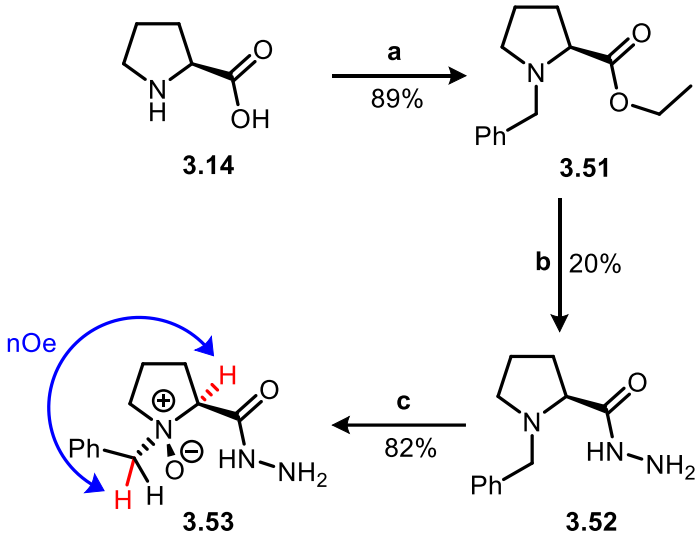
3.2.4.3. Oxidation of *N*-Benzyl-*L*-Proline Hydrazide

Another area of study in the past has been the introduction of multiple H-bond donors into the proline side chain. Previously it was shown that a 6-membered intramolecular hydrogen bond was favoured over the more remote 10-membered hydrogen bond offered in a dipeptide side chain.[146, 147] We wished to investigate the effect of having more proximate H-bond donors, by introducing a hydrazide group to *L*-proline. This would offer the option of a 6- or 7-memebered ring H-bond interaction between the *N*-oxide and the side chain NHs.

The *N*-benzyl-*L*-proline hydrazide **3.53** was synthesised from proline, following a literature procedure, shown in Scheme 3.24.[148] Esterification of *L*-proline was achieved with thionyl chloride in ethanol, to give *L*-proline ethyl ester. This was immediately *N*-benzylated with benzyl bromide in the presence of potassium carbonate and acetone, affording **3.51** in 89% overall yield. *N*-Benzyl-*L*-proline ethyl

ester **3.51** was then treated with hydrazine monohydrate in methanol to give the *N*-benzyl-*L*-proline hydrazide **3.52** in a relatively poor 20% yield. Two distinct NH peaks were seen in the ^{1}H NMR at 8.27 ppm, for the amide NH and 3.75 ppm for the terminal NH_2.

Oxidation of **3.52**, using *m*-CPBA in DCM, gave the *N*-benzyl-*L*-proline hydrazide *N*-oxide **3.53** in an 82% yield. *N*-Oxide formation was confirmed by HRMS, with the downfield shifting of all proximal protons also being seen. There was a significantly large downfield shift of the amide NH signal, from 8.27 ppm to 11.94 ppm. This indicates that there is a hydrogen bonding interaction taking place between the *N*-oxide and this proton. There was also a downfield shift of the terminal NH_2 protons, but not to the same magnitude.

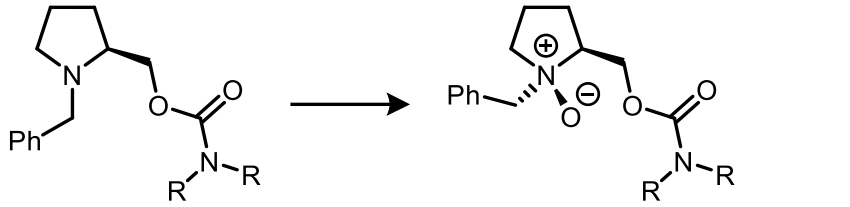


Scheme 3.24: *Reagents and Conditions* **a-** (i) $SOCl_2$ (1.0 eq.), EtOH, reflux (ii) BnBr (1.5 eq.) K_2CO_3 (2.0 eq.), acetone, o/n **b-** $NH_2NH_2.H_2O$ (2.0 eq.), MeOH, 2 days **c-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

N-Oxide **3.53** formed as a single diastereoisomer, as confirmed by ^{13}C NMR. Oxidation occurred entirely *syn* to the hydrazide side chain. This was confirmed by an nOe observed between the C-*α* and benzylic protons, marked red in Scheme 3.24.

3.2.5. Hydrogen Bond Donor Requirement Testing

The *N*-oxidation of *N*,*N*-dialkyl carbamate derivatives **3.38**, possessing no directing hydrogen bond donor in the pyrrolidine side chain, to their corresponding *N*-oxides **3.39** (Scheme 3.25) proceeded with complete diastereoselectivity, discussed in more detail in Section 3.2.3.. These results were contrary to previous results, discussed in Section 3.1.1., when the absence of a hydrogen bond donor in the side chain of these systems removed the selectivity of *N*-oxidation.

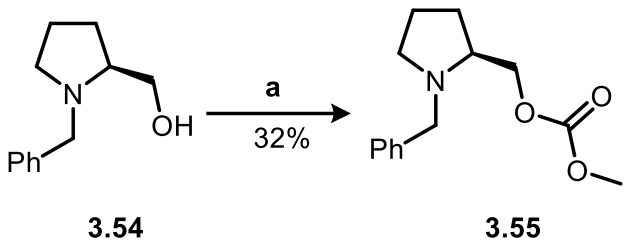


3.38 **3.39** R = Me or ^{i}Pr

Scheme 3.25: *N*-Oxidation of *N*,*N*-dialkyl carbamate derivatives to their corresponding *N*-oxides.

We wished to investigate the cause of the observed *N*-oxidation selectivity being maintained in the absence of any H-bond donor in these adducts. We wished to synthesise a series of *N*-benzyl proline derivatives with sequentially shortened side chains, from a carbonate to methyl ether analogues, all possessing no hydrogen bond donor. The selectivity, if any, of tertiary amine *N*-oxidation would then be observed by oxidation under the established conditions.

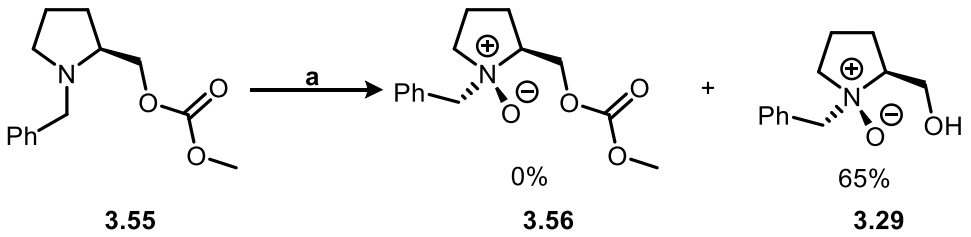
At first a carbonate group was introduced to the *N*-benzyl proline system, shown in Scheme 3.26. The carbonate **3.55** was synthesised by deprotonation of *N*-benzyl prolinol **3.54** with an excess of pyridine in THF followed by addition of methyl chloroformate, which proceeded in a low 32% yield. A clear carbonate carbonyl peak at 155.9 ppm in the ^{13}C NMR was observed along with a distinct carbonyl stretch at 1744 cm^{-1} in the IR spectrum, confirming carbonate formation.



3.54 **3.55**

Scheme 3.26: Preparation of carbonate pyrrolidine side chain. *Reagents and Conditions* **a-** (i) pyridine (3.0 eq.), THF, 0 °C, 30 mins (ii) MeO_2CCl (3.0 eq.), o/n.

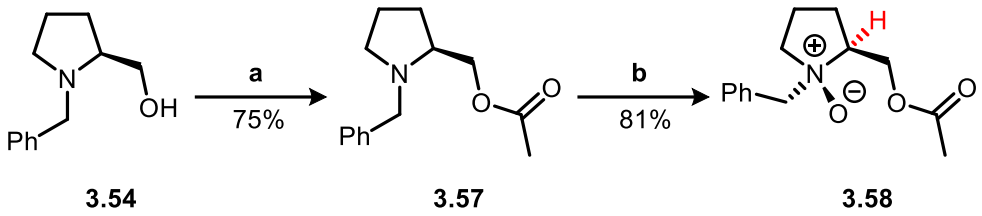
Treatment of tertiary amine **3.55** with *m*-CPBA in DCM at -78°C yielded no *N*-oxide **3.56** (Scheme 3.27). Instead, *N*-benzyl prolinol *N*-oxide **3.29** was the lone isolated product from the reaction, which by comparison to a pure sample made by direct oxidation of *N*-benzyl prolinol, was confirmed to have the geometry shown in Scheme 3.27. It was not apparent as to whether *N*-oxidation or carbonate decomposition was taking place first. What became clear was decomposition occurred rapidly, as periodic NMRs of crude reaction mixtures showed no presence of the desired *N*-oxide product **3.56**. One possible explanation for this result is that it is possible the decomposition of the carbonate was driven by the *meta*-chlorobenzoic acid produced from the oxidation of the tertiary amine species, leading to acid catalysed hydrolysis of the carbonate bond to give the hydroxyl product **3.29**.



3.55 **3.56** **3.29**

Scheme 3.27: *Reagents and Conditions* **a-** *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

Having been unable to obtain the carbonate *N*-oxide analogue it was decided to shorten the pyrrolidine side chain further. To achieve this an acetate side chain was incorporated into the pyrrolidine side chain (Scheme 3.28), which would remove any effect the nitrogen of the carbamate may be having on *N*-oxidation selectivity. The acetate group was added by treating *N*-benzyl prolinol **3.54** with acetyl chloride in the presence of triethylamine to give acetate **3.57** in a 75% yield.



Scheme 3.28: Acetate pyrrolidine side chain *N*-oxide synthesis. *Reagents and Conditions* **a**- NEt_3 (1.1 eq.), acetyl chloride (1.1 eq.), DCM, 0°C, 5h **b**- *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

N-Oxidation of tertiary amine **3.57** with *m*-CPBA gave the *N*-oxide species **3.58** in an 81% yield. This displayed the characteristic downfield shift of the *α*-proton, shown in red, from 2.88 ppm to 3.96 ppm indicating *N*-oxide formation. The *N*-oxide **3.58** was obtained as a single diastereoisomer, with no minor product observed even when the reaction was also followed by NMR to completion. The diastereoselectivity of tertiary amine oxidation was defined by a series of nOe experiments. Irradiation of the *α*-proton showed through space interactions with the benzylic CH_2, only possible if both exist on the same face of the compound which confirmed oxidation had occurred exclusively *syn* to the side chain (Figure 3.8). Acetate decomposition was observed when stored at ambient temperature for long periods of time, but freezer storage of **3.58** significantly extended this.

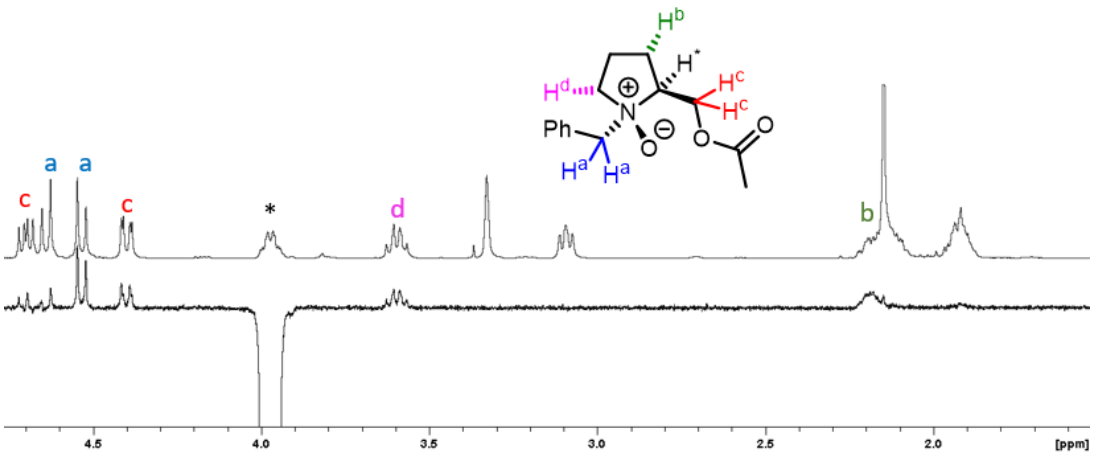
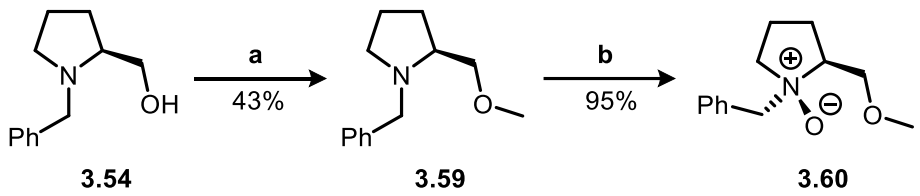


Figure 3.8: nOe interaction of acetate derivative **3.58** irradiating the *α*-proton, H^*.

With *N*-oxidation selectivity being maintained with an acetate side chain on the pyrrolidine ring, we examined the oxidation of a prolinol methyl ether derivative **3.59**, this would remove any influence the carbonyl was having on the oxidation process. The methyl ether adduct **3.59** was synthesised by deprotonation of *N*-benzyl prolinol with NaH and subsequent addition of methyl iodide, following an analogous literature procedure.[149] Oxidation of amine **3.59**, with *m*-CPBA in DCM, gave the *N*-oxide species **3.60** in a 95% yield.



Scheme 3.29: Methyl ether pyrrolidine side chain *N*-oxide synthesis. *Reagents and Conditions* **a**- (i) NaH (2.0 eq.), THF, 0°C, 1h (ii) MeI (1.0 eq.), TBAI (cat.), o/n **b**- *m*-CPBA (1.2 eq.), K_2CO_3 (1.5 eq.) DCM, -78°C, o/n.

Once again the characteristic *α*-proton downfield shift was observed, from 2.71 ppm in **3.59** to 3.83 ppm in the *N*-oxide *α*-proton **3.60**. As with the acetate **3.58** and the dialkyl carbamates **3.39** the methyl ether *N*-oxide **3.60** was formed as a single diastereoisomer. Again nOe experiments were utilised to assign the diastereoselectivity of the product. Irradiation of the C-*α* proton again established a through space interaction with the benzylic CH_2, marked 'a' in Figure 3.9, meaning oxidation must have selectively take place on the top face of the system again giving the *syn N*-oxide. Somewhat surprisingly the *N*-oxide **3.60** was highly stable, being stored for prolonged periods of time in the fridge with no observable degradation.

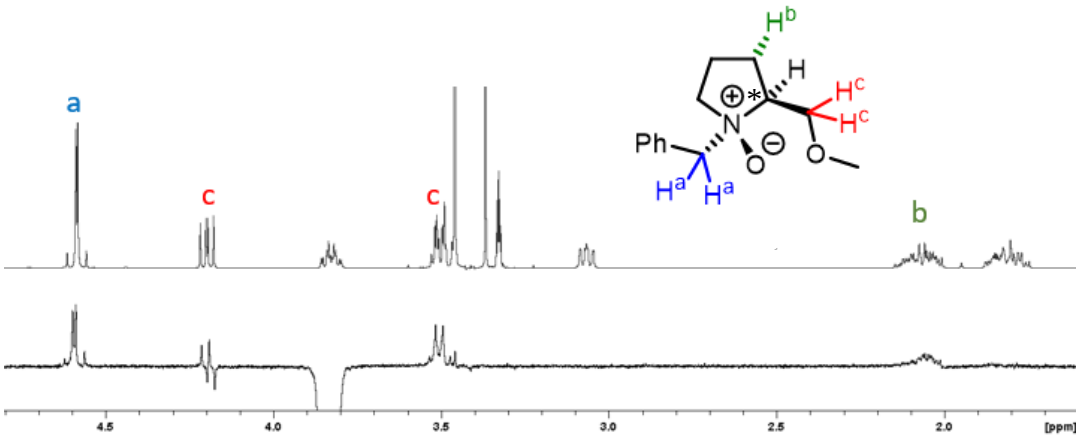
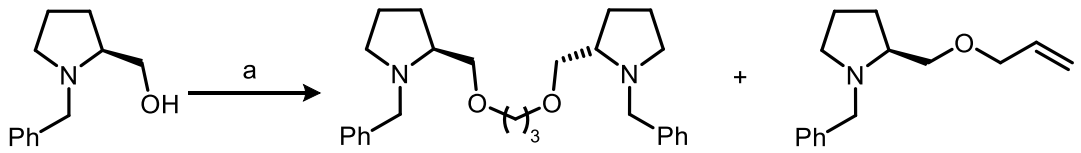


Figure 3.9: nOe interaction of methoxy derivative **3.60** irradiating the α-proton, H^*.

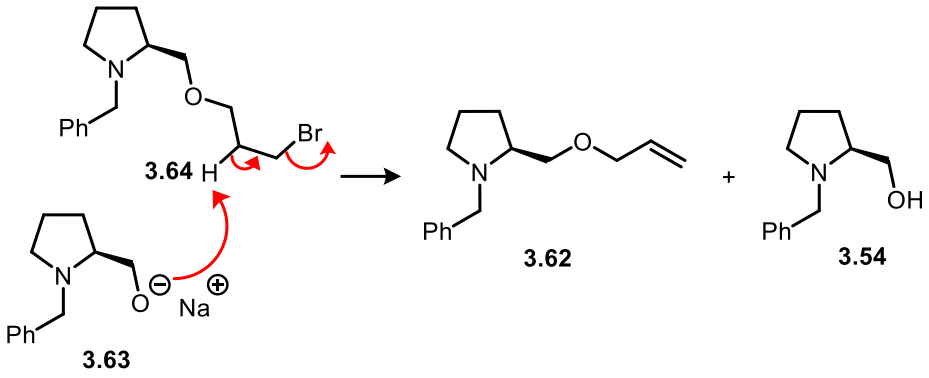
We wished to synthesise a dimeric analogue of the *N*-benzyl-*L*-prolinol methyl ether *N*-oxide. We hypothesised linking the two molecules of *N*-benzyl prolinol *via* an alkyl liker, attached to the hydroxyl terminus, which could then undergo oxidation to the bis-*N*-oxide species. *N*-Benzyl-*L*-prolinol **3.54** was deprotonated with NaH in THF at 0 °C followed by addition of 1,3-dibromopropane. None of the desired bis-amine **3.61** was isolated from the reaction, instead an alternative product was isolated along with almost half of the *N*-benzyl prolinol starting material (Scheme 3.30). The alternative product was identified as the *O*-allyl amine **3.62**, with distinct vinylic peaks at 5.90, 5.29 and 5.18 ppm.



Scheme 3.30: Methyl ether *N*-oxide dimer synthesis. *Reagents and Conditions* **a**-(i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) 1,3-dibromopropane (0.5 eq.), o/n**.**

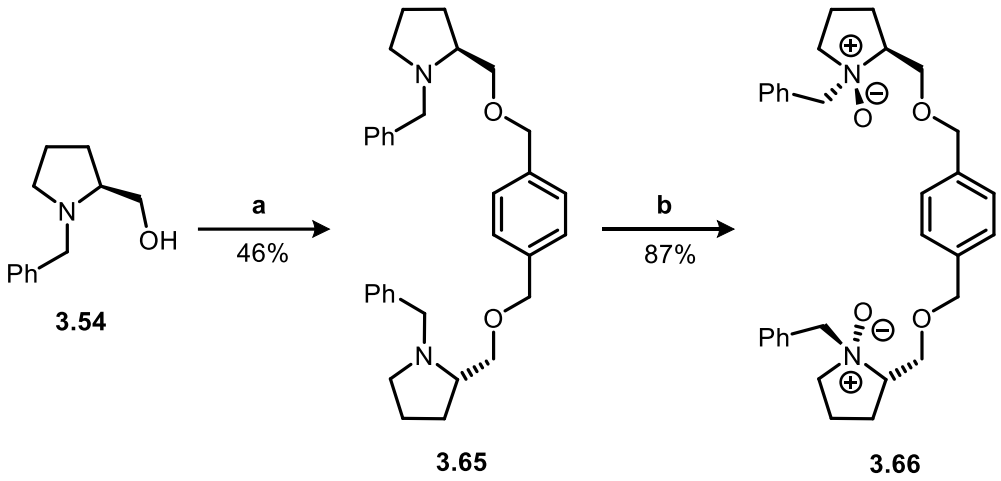
The *O*-allyl amine **3.62** by-product which was presumably the result of an E2 elimination of the mono addition product **3.64**, as shown in Scheme 3.31. The single

O-alkylation product **3.64** is first formed by halogen displacement by one alkoxide anion, which then undergoes E2 elimination driven by a second molecule of the alkoxide **3.63**, regenerating **3.54**, explaining the high recovery of starting material.



Scheme 3.31: E2 elimination to give *O*-allyl by-product

An alternative method to incorporate the ether linkage involved a xylene linking group instead of the alkyl chain linker, which would block the possibility of the E2 elimination reaction (Scheme 3.32). *N*-Benzyl-*L*-prolinol was treated with NaH in THF, followed by addition of *p*-xylylene dibromide to yield the phenyl ether linked bis-amine **3.65** in a 46% yield. *N*-Oxidation with *m*-CPBA in DCM gave the bis-*N*-oxide species **3.66** in an 87% yield.



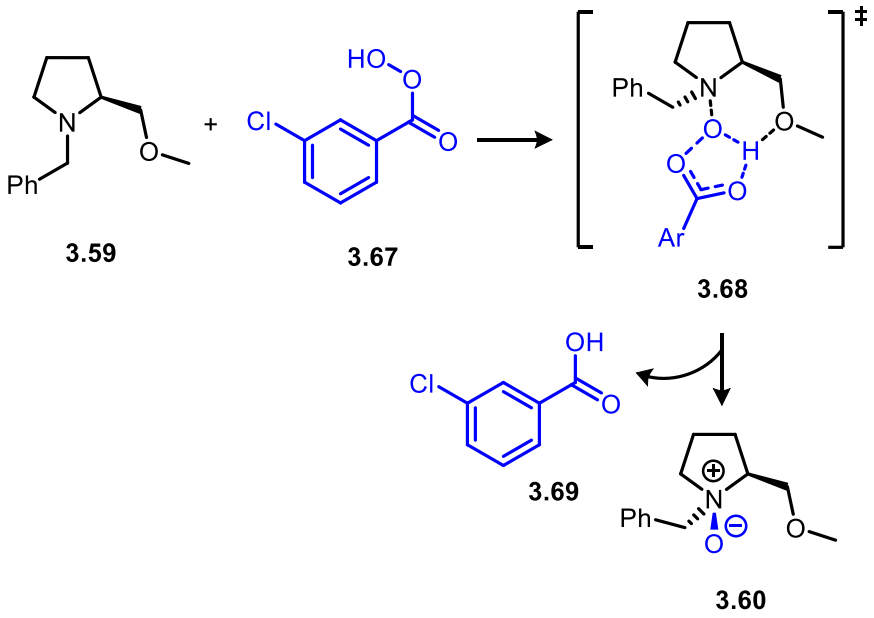
Scheme 3.32: Methyl ether *N*-oxide dimer synthesis. *Reagents and Conditions* **a**- (i) NaH (1.2 eq.), THF, 0°C, 30 mins (ii) *p*-xylylene dibromide (0.5 eq.), o/n **b**- *m*-CPBA (2.2 eq.), K_2CO_3 (2.5 eq.) DCM, -78°C, o/n.

In accordance with results observed in the oxidation of the methyl ether analogue **3.59**, the closest related structure, the *N*-oxidation of **3.65** to bis-*N*-oxide **3.66** proceeded with complete *syn*-selectivity.

3.2.6. Alternative Reasons For *syn*-Stereoselectivity

The results discussed in Section 3.2.5. clearly show that diastereoselectivity of the *N*-oxidation of these types systems is not reliant on the presence of a H-bond donor. Previous work has been directed on the basis that a hydrogen bond donor is required, guiding delivery of the oxygen by intramolecular interactions with the incoming *m*-CPBA. These H-bond donors can also offer a stabilising effect by forming intramolecular H-bonds with the newly formed *N*-oxide. However, most of the examples described where the hydrogen bond has been removed in the side chains have proven to be indefinitely stable. Also, the *N*-benzyl-*L*-prolinol carbamate *N*-oxides exist with no observable intramolecular H-bonding, yet are also highly stable structures.

With the results discussed in this chapter taken into account, an alternative driving force for *N*-oxidation selectivity must be in effect. One common feature shared by all analogues in this work is the presence of an oxygen or nitrogen at the γ-position to the tertiary amine nitrogen. It seems reasonable to suggest that the pyrrolidine side chain is actually acting as the hydrogen bond acceptor rather than donor, in the oxidation of these tertiary amines. As shown in Scheme 3.33 oxidation of the methyl ether **3.59** with *m*-CPBA **3.67** could be directed by hydrogen bonding of the ether oxygen to the peracid proton. This could lead to the transition state (TS) **3.68** with the ether oxygen guiding the *N*-oxidation *syn* to the ether side chain. A similar TS could be drawn for all other examples discussed in this chapter.



Scheme 3.33: Alternative suggested *N*-oxidation transition state with *m*-CPBA

Alternatively, sterics of the *N*-benzyl-*L*-proline derived tertiary amines could be a driving force for the observed selectivity. Although more flexible than their 6-membered counterparts, 5-membered ring substituents will still adopt pseudo-axial and pseudo-equatorial positions. Crucially, in the case of the *N*-benzylated systems being used in this work, the lone pair of the nitrogen can exist on the top or bottom face of the pyrrolidine ring system (Figure 3.10), which would be determined by the relative energy difference between the two forms.

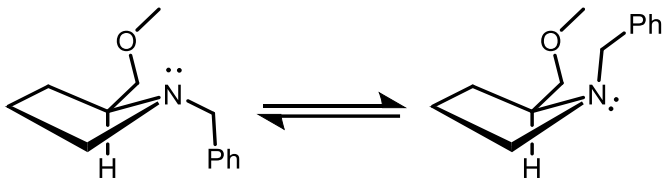


Figure 3.10: lone pair possible positions in methyl ether derivative **3.59**.

Figure 3.11 shows the lowest energy conformation of the *N*-benzyl-*L*-prolinol methyl ether derivative, calculated using the ωB97X-D functional and 6-31G* basis set, in which both the methyl ether side chain and *N*-benzyl group are positioned pseudo-equatorialy. This positions the lone pair of the tertiary amine nitrogen, required for attack of the peracid, on the 'top' face of the ring. Due to this lowest energy conformation, and lone pair positioning, peracid oxidation could favour *syn* over the *anti*-oxidation. On the other hand, it is work stating that according the Curtin-

Hammett principles the reaction does not necessarily have to react *via* the lowest energy conformation of the starting material. Therefore, without more in depth studies of the transition state of the reaction this reasoning for complete oxidation diastereoselectivity cannot be concluded as the sole controlling factor.

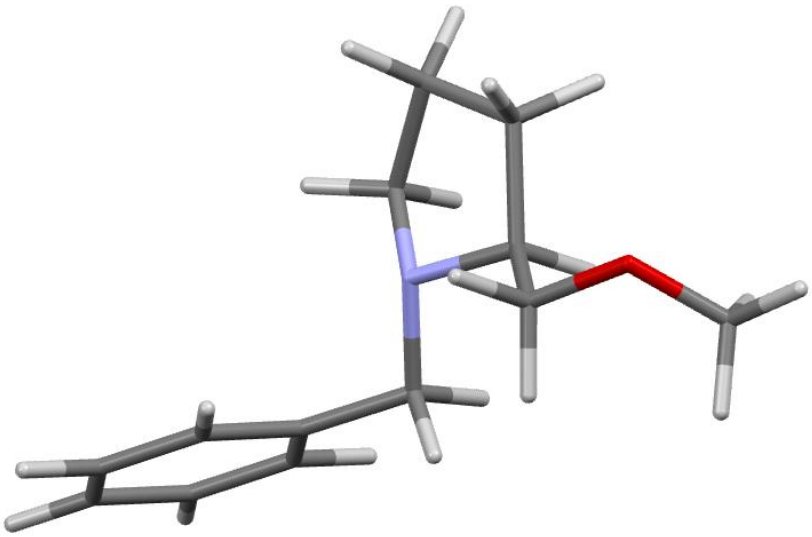
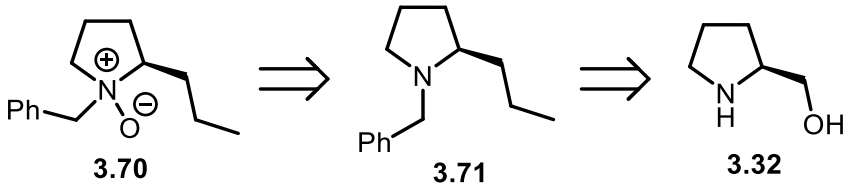


Figure 3.11: lowest energy conformation of *N*-benzyl-*L*-prolinol methyl ether **X**.

It is worth noting that neither of the proposals presented above have been shown to be behind the apparent *N*-oxidation selectivity seen across all derivatives at this time. Moreover, the loss of *N*-oxidation selectivity of the *N*-benzyl-*L*-proline esters is not explained by either of these reasons. Work is ongoing to fully understand the mechanism behind the selectivity of oxidation in the absence of a hydrogen bond donor. Currently this work involves complete removal of all heteroatoms from the pyrrolidine ring side chain to give the propyl derivative **3.71**, shown in Scheme 3.34, of which oxidation will be controlled purely by the sterics of the system and transition state energy requirements.



3.70 **3.71** **3.32**

Scheme 3.34: Proposed retrosynthetic route to *N*-benzyl proline propyl *N*-oxide derivative **3.70** from prolinol.

3.3. Conclusions and Future Work

A variety of chiral *N*-benzyl-*L*-proline derived tertiary amine *N*-oxides have been synthesised. The scope of pyrrolidine side chain functionality has been investigated and expanded on from previous work within the group. Carbamate, hydroxamic acid, hydrazide and oxime groups have been incorporated into the *N*-benzyl proline derived systems. *N*-Oxidation of all systems with side chains containing H-bond donors proceeded with complete *syn* diastereoselectivity.

In the case of the carbamate derivatives, homochiral bis-*N*-oxide species were synthesised, by linking *via* the pyrrolidine amine or carbamate chain. These structures bare strong similarities to structures currently being used as asymmetric catalysts. For this reason, these *N*-oxides, and derivatives thereof, can be used in screened as ligands for catalysts.

Studies into H-bond donor requirement for *N*-oxidation facial selectivity were also carried out. This found that *syn*-selectivity was maintained even when the Hydrogen bond donor groups were concealed.

Investigations are ongoing as to the exact reason for maintenance of *N*-oxidation facial selectivity in the absence of a H-bond donor. This currently entails removal of all heteroatoms from the chain, testing the hypothesis that the presence of a H-bond acceptor causes the selectivity.

Chapter 4

Experimental Section

4. Experimental Section

4.1. General Experimental Details

4.1.1. Chemicals

Unless stated, all materials were purchased from commercial sources (Sigma Aldrich, Alfa Aesar and Fluorochem) and used without any further treatment. Reagents requiring purification were purified using standard laboratory techniques according to methods published by Perrin, Armarego, and Perrin (Pergamon Press, 2009).[150]

4.1.2. Solvents

Anhydrous solvents were obtained *via* solvent passage through drying columns supplied by MBraun (MB-SPS-800), or were purchased in Sure/Seal™ bottles from Sigma Aldrich. Solvents were removed from the reaction mixtures with the use of rotary evaporators connected to vacuum lines.

4.1.3. Column Purification and TLC

Thin layer chromatography was performed using Merck Kieselgel 60 F_{254} aluminium backed silica plates. Visualization was achieved by UV fluorescence or a basic $KMnO_4$ solution and heat. Flash column chromatography (FCC) was performed using silica gel (Aldrich 40-63 μm, 230-400 mesh). The crude material was pre-adsorbed onto silica prior to application to the column.

4.1.4. Spectral Data Collection

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III HD 400 MHz or a Bruker Avance III HD 500 MHz spectrometer. Chemical shifts (δ) are given in parts per million (ppm). Peaks are described as singlets *(S)*, doublets (d), triplets (t), quartets (q), doublet of doublets (dd), doublet of triplets (dt), triplet of doublets (td), multiplets (m), broad (br.) and apparent (app.). Coupling constants (*J*) are quoted to the nearest 0.5 Hz. Most assignments of NMR spectra were based on 2D NMR data (COSY and HSQC). Mass spectra were recorded using a micromass LCT mass spectrometer (ES+) or an Agilent QTOF 7200 mass spectrometer (CI). Infrared spectra were recorded on a Perkin Elmer 1720-x FT-IR spectrometer as thin films or solids compressed on a diamond plate.

The numbering of compound structures does not necessarily reflect the numbering contained in the systematic names.

4.2. General Procedures

4.2.1. General Procedure A

To a stirred solution of acid (1.0 eq.) in DCM (10 mL/mmol) was added oxalyl chloride (1.5 eq.) dropwise and DMF (cat.) at 0 °C. The reaction mixture was allowed to stir at rt until TLC indicated no starting material remained and solvents were removed *in vacuo*. The pale yellow residue was taken back up DCM (10 mL/mmol). To this solution RNH_2 (1.0 eq.) and DIPEA (1.0 eq.) were added, and the reaction mixture was allowed to stir for 18 h at rt. Solvents were removed *in vacuo* and the crude material was purified by FCC (eluting with EtOAc:MeOH) to afford the title compound.

4.2.2. General procedure B

To a stirred solution of *N*-benzyl-prolinol (1.0 eq.) in THF (5 mL/mmol) at 0 °C was added NaH (60% dispersion in mineral oil, 1.1 eq.). The reaction mixture was allowed to stir at 0 °C for 0.5 hours followed by the addition of isocyanate (1.1 eq.). The reaction mixture was allowed to warn to ambient temperature and stirred for a further 18 hours. The reaction was quenched with MeOH and solvent removed *in vacuo*. The resulting residue was taken up in DCM and H_2O and extracted with DCM (x 2), combined organic extracts dried ($MgSO_4$) and solvents removed *in vacuo*. The residue was purified by FCC (eluting with EtOAc:hexanes) to give title compound.

4.2.3. General procedure C

To a stirred solution of *N*-benzyl-prolinol derivative (1.0 eq.) in DCM (10 mL/mmol) at -78 °C was added *m*-CPBA (1.2 eq.) and K_2CO_3 (1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were then removed *in vacuo* and the residue was purified by FCC (eluting with EtOAc:MeOH) to afford the title compound.

4.2.4. General procedure D

To a stirred solution of *N*-benzyl-prolinol *N*-oxide (1.0 eq.) in THF (5 mL/mmol) at 0 °C was added NaH (60% dispersion in mineral oil, 1.1 eq.). The reaction mixture was allowed to stir at 0 °C for 0.5 hours followed by the addition of isocyanate (1.1 eq.). The reaction mixture was allowed to warn to ambient temperature and stirred for a further 18 hours. The reaction was quenched with MeOH and solvent removed *in*

vacuo. The resulting residue was taken up in DCM and H_2O and extracted with DCM (x 2), combined organic extracts dried ($MgSO_4$) and solvents removed *in vacuo*. The residue was purified by FCC (eluting with EtOAc:MeOH) to give the title compound.

4.2.5. General Procedure E

Triethylamine was degassed with N_2 before being used in the reaction. Aryl iodide (1.0 eq.), CuI (0.04 eq.) and bis(triphenylphosphine)palladium (II) dichloride (0.01 eq.) were added to *(S)*-3-(2-((prop-2-yn-1-yloxy)methyl)pyrrolidin-1-yl)propanenitrile (1 eq.) in degassed triethylamine (4 mL/mmol) at room temperature. The reaction was followed to completion by TLC (eluting with EtOAc). Upon completion solvents were removed *in vacuo*, the residue was taken up in EtOAc and H_2O, and the product was extracted with EtOAc (x3). The combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with EtOAc:hexanes) to afford the title compound.

4.2.6. General Procedure F

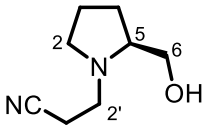
To a stirred solution of amine (1.0 eq.) in DCM (10 mL/mmol) at -78 °C was added *m*-CPBA (1.2 eq.) and K_2CO_3 (1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were then removed *in vacuo* and the residue was purified by FCC (eluting with EtOAc:MeOH) to afford the title compound.

4.2.7. General Procedure G

To a stirred solution of enamine *N*-oxide (1.0 eq.) in MeOH (10 mL/mmol) was added 10% Pd/C (0.05 eq.), and the reaction mixture was allowed to stir for 18 h under a H_2 atmosphere. The reaction mixture was filtered through a pad of Celite® and solvents removed *in vacuo* and the residue was purified by FCC (eluting with EtOAc:hexanes) to afford the title compound.

4.3. Individual Experimental Details - for Chapter 2

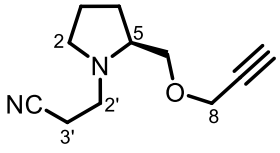
4.3.1. Preparation of *N*-Cyanoethyl Prolinol (2.6)



Acrylonitrile (8.36 mL, 118.6 mmol, 1.2 eq.) was added dropwise to a solution of prolinol (10.00 g, 98.9 mmol, 1.0 eq.) in methanol (200 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and allowed to stir for a further 18 h. After this time TLC showed no remaining starting prolinol. Solvents and excess acrylonitrile were removed *in vacuo* to give the title compound as a pale yellow oil which was used with no further purification.

v max/ cm^{-1} : 3417 (O-H), 2247 (C≡N) and 1081 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (dd, 1H, *J* = 11.0, 3.5 Hz, C**6**-H), 3.42 (dd, 1H, *J* = 11.0, 2.5 Hz, C**6**-H), 3.21 (m, 1H, C**2**-H), 3.07 (dt, 1H, *J* = 12.5, 7.5 Hz, C**2'**-H), 2.71 (m, 1H, C**5**-H), 2.65 (dd, 1H, *J* = 12.0, 6.0 Hz, C**2'**-H), 2.53 (m, 2H, C**3'**-CH_2), 2.48 (br. S, 1H, OH), 2.34 (dd, 1H, *J* = 16.5, 8.5 Hz, C**2**-H), 1.89 (m, 1H, C**4**-H), 1.78 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 118.8 (CN), 64.6 (C**5**), 62.5 (C**6**), 54.1 (C**2**), 49.9 (C**2'**), 27.4 (C**4**), 23.8 (C**3**), 18.2 (C**3'**); HRMS: (CI^+) calculated for $C_8H_{14}N_2O$: 155.1179. Found $[M+H]^+$: 155.1186.

4.3.2. Preparation of *(S)*-3-(2-((prop-2-yn-1-yloxy)methyl)pyrrolidin-1-yl)propanenitrile (2.19)

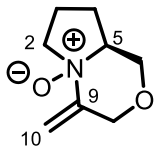


NaH (60% dispersion in mineral oil, 1.56 g, 38.9 mmol, 1.2 eq.) was added in portions to a stirred solution of *(S)*-3-(2-(hydroxymethyl)pyrrolidin-1-yl)propanenitrile **2.6** (5.00 g, 32.4 mmol, 1.0 eq.) in THF (100 mL) under a N_2 atmosphere at 0 °C. The reaction mixture was allowed to stir for 0.5 hours at 0 °C followed by dropwise

addition of propargyl bromide (80% wt. in toluene, 4.34 mL, 38.9 mmol, 1.2 eq.). The reaction was allowed to warm to ambient temperature and was monitored by TLC. Upon complete disappearance of starting material the reaction was quenched with methanol and solvents were removed *in vacuo* to give a cloudy yellow residue. The residue was taken up in EtOAc (200 mL) and washed with sat. sodium bicarb. solution (150 mL) and brine (150 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:1 EtOAc:hexanes) to afford the title compound (4.97 g, 80%) as a yellow oil.

ν_{max}/cm^{-1}: 3284 (C-H), 2968 and 2874 (C-H) and 2247 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 4.17 (m, 2H, C**8**-CH_2), 3.49 (m, 2H, C**6**-CH_2), 3.19 (m, 2H, C**2**-H and C**2'**-H), 2.71 (m, 2H, C**5**-H and C**2'**-H), 2.52 (t, 2H, *J* = 7.5 Hz, C**3'**-CH_2), 2.43 (t, 1H, *J* = 2.5 Hz, C**10**-H), 2.33 (app. q, 1H, *J* = 8.5 Hz, C**2**-H), 1.90 (m, 1H, C**4**-H), 1.76 (m, 2H, C**3**-H), 1.60 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$, Me_4Si) δ 119.1 (CN), 79.7 (C**9**), 74.5 (C**10**), 73.5 (C**6**), 62.9 (C**5**), 58.5 (C**8**), 54.4 (C**2**), 50.6 (C**2'**), 28.2 (C**4**), 23.2 (C**3**), 17.7 (C**3'**); HRMS: (CI^+) calculated for $C_{11}H_{15}N_2O$: 193.1335. Found $[M+H]^+$: 193.1343.

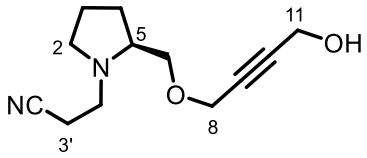
4.3.3. Preparation of (8a*S*)-4-methylenehexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.20)



To a stirred solution of *N*-cyanoethyl alkyne **2.19** (0.2 g, 10.4 mmol, 1.0 eq.) in DCM (15 mL) at -78 °C was added *m*-CPBA (0.20 g, 1.15 mmol, 1.1 eq.) and K_2CO_3 (0.22 g, 1.56 mmol, 1.5 eq.). The reaction was allowed to slowly warm to ambient temperature and stirred overnight. The reaction mixture was then filtered and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:1 EtOAc:MeOH) to afford the title compound (015 g, 92%) as a pale yellow semi solid.

v max /cm^{-1}: 2969 (C-H), 1672 (C=C), 1369 (C-N) and 1107 (C-O); ^{1}H NMR (400 MHz, CD_3OD) δ 6.00 (s, 1H, C**10**-H), 5.22 (s, 1H, C**10**-H), 4.67 (d, 1H, *J* = 13.5 Hz, C**8**-H), 4.18 (m, 1H, C**6**-H), 4.15 (d, 1H, *J* = 13.5 Hz, C**8**-H), 4.04 (ddd, 1H, *J* = 12.0, 8.5, 6.0 Hz, C**2**-H), 3.68 (m, 1H, C**5**-H), 3.54 (dd, 1H, J = 13.0, 7.0 Hz, C**6**-H), 3.42 (m, 1H, C**2**-H), 2.49 (m, 1H, C**4**-H), 2.40 (m, 1H, C**3**-H), 2.97 (m, 1H, C**3**-H), 1.85 (m, 1H, C**4**-H); ^{13}C NMR (100 MHz, CD_3OD) δ 148.1 (C**9**), 108.9 (C**10**), 77.7 (C**5**), 66.6 (C**6**), 66.3 (C**8**), 65.3 (C**2**), 23.3 (C**4**), 19.7 (C**3**); HRMS: (CI^{+}) Calculated for $C_8H_{13}NO_2$ [M+H]$^{+}$: 156.0964. Found [M+H]$^{+}$: 156.1025.

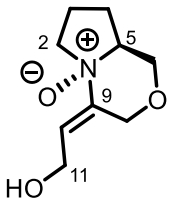
4.3.4. Preparation of *(S)*-3-(2-(((4-hydroxybut-2-yn-1-yl)oxy)methyl)pyrrolidin-1-yl)propanenitrile (2.27)



To a stirred solution of propargyl ether **2.19** (1.0 g, 5.20 mmol, 1.0 eq.) in DMSO (4 mL) was added Triton B (40% wt. in MeOH, 0.22 mL, 0.52 mmol, 0.1eq.) and paraformaldehyde (0.19 g, 6.25 mmol, 1.2 eq.). The reaction was allowed to stir for 18 hours and then diluted with EtOAc (40 mL). The organic phase was washed with H_2O (40 mL) sat. sodium bicarb. solution (40 mL), brine (40 mL). The organic phase was dried ($MgSO_4$) and reduced *in vacuo.* The crude material was purified by FCC (eluting with 4:1 EtOAc:hexanes) to afford the title compound (0.66 g, 57%) as a pale yellow oil.

v max /cm^{-1}: 3395 (O-H), 2248 (C≡N), 1619 (C≡C) and 1020 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (t, 2H, *J* = 2.0 Hz, C**11**-CH_2), 4.20 (m, 2H, C**8**-CH_2), 3.53 (dd, 1H, *J* = 9.5, 6.5 Hz, C**6**-H), 3.47 (dd, 1H, *J* = 9.5, 4.5 Hz, C**6**-H), 3.25 (m, 1H, C**2'**-H), 3.17 (m, 1H, C**5**-H), 2.75 (m, 1H, C**2**-H), 2.69 (m, 1H, C**2'**-H), 2.53 (app. t, 2H, *J* = 7.0 Hz, C**6**-H), 2.31 (dd, 1H, *J* = 16.5, 9.0 Hz, C**2**-H), 2.17 (s, 1H, OH), 1.90 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-H and C**4**-H), 1.57 (m, 1H C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 119.3 (CN), 85.0 (C**9**), 81.5 (C**10**), 73.7 (C**6**), 63.0 (C**5**), 58.7 (C**8**), 54.5 (C**2**), 50.9 (C**2'**), 50.6 (C**11**), 28.2 (C**4**), 23.1 (C**3**), 17.8 (C**3'**); HRMS: (ESI^{+}) Calculated for $C_{12}H_{18}N_2O_2$: 223.1441. Found [M+H]$^{+}$: 223.1450.

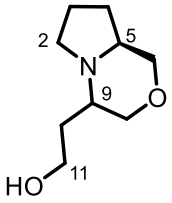
4.3.5. Preparation of (8a*S*,*E*)-4-(2-hydroxyethylidene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.29)



To a stirred solution of tertiary amine **2.27** (0.63 g, 2.84 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.59 g, 3.40 mmol, 1.2 eq.) and K_2CO_3 (0.59 g, 4.25 mmol, 1.5 eq.). The reaction was allowed to slowly return to ambient temperature and stirred overnight. The reaction was filtered and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:1 EtOAc:MeOH) to afford the title compound (0.40 g, 76%) as a pale yellow powder.

v max /cm^{-1}: 3362 (O-H) and 1107 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93 (t, 1H, *J*=6.0 Hz, C**10**-H), 4.83 (d, 1H, *J*=14.0 Hz, C**8**-H), 4.27 (m, 1H, C**11**-H), 4.13 (d, 1H, *J*=14.0 Hz, C**8**-H), 4.01 (m, 1H, C**6**-H), 3.97 (m, 1H, C**2**-H), 3.73 (tt, 1H, *J*=8.5, 4.0 Hz, C**5**-H), 3.43 (dd, 1H, *J*=13.0, 4.0, C**6**-H), 3.33 (m, 1H, C**2**-H), 2.54 (m, 1H, C**4**-H), 2.44 (m, 1H, C**3**-H), 1.98 (m, 1H, C**3**-H), 1.70 (m, 1H, C**4**-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.9 (C**9**), 126.8 (C**10**), 77.5 (C**5**), 67.0 (C**6**), 65.2 (C**2**), 61.9 (C**8**), 57.5 (C**11**), 24.0 (C**4**), 20.2 (C**3**); HRMS: (CI^+) Calculated for $C_9H_{16}NO_3$: 186.1130. Found $[M+H]^+$: 186.1125.

4.3.6. Preparation of 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)ethan-1-ol (2.30)

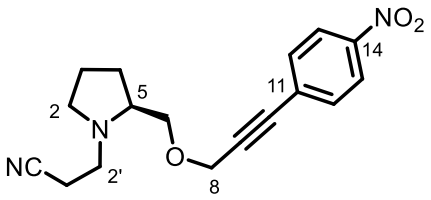


To a stirred solution of enamine *N*-oxide **2.29** (0.30 g, 1.62 mmol, 1.0 eq.) in MeOH (15 mL) under a nitrogen atmosphere was added Pd/C (10% wt., 0.07 g, 0.16 mmol, 0.1 eq.). The reaction was placed under a H_2 atmosphere and followed by TLC. Upon

completion of the reaction the solvent were removed *in vacuo* and the crude material was purified by FCC (eluting with 4:1 EtOAc:MeOH) to afford the title compound (0.28 g, 69%) as a pale yellow oil.

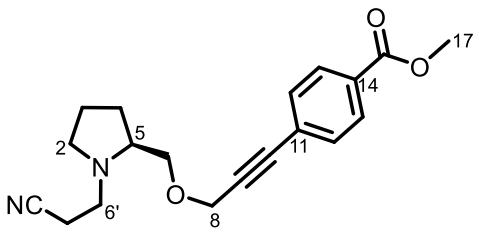
v max /cm^{-1}: 3298 (O-H,) and 1126 (C-O); ^{1}H NMR (400 MHz, CH_3OD) δ 3.96 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.91 (dd, 1H, *J* = 11.5, 3.0 Hz, C**8**-H), 3.62 (t, 2H, *J* = 6.5 Hz, C**11**-CH_2), 3.34 (dd, 1H, *J* = 9.0, 5.0 Hz, C**2**-H), 3.29 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 3.20 (app. t, 1H, *J* = 11.5 Hz, C**8**-H), 2.51 (m, 1H, C**9**-H), 2.30 (m, 1H, C**5**-H), 2.20 (q, 1H, *J* = 9.0 Hz, C**2**-H), 1.89 (m, 2H, C**10**-H and C**4**-H), 1.80 (m, 2H, C**3**-CH_2), 1.51 (m, 1H, C**10**-H), 1.39 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, CH_3OD) δ 70.2 (C**6**), 69.5 (C**8**), 62.9 (C**5**), 60.3 (C**9**), 58.4 (C**11**), 50.2 (C**2**), 32.6 (C**10**), 25.1 (C**4**), 19.7 (C**3**); HRMS: (CI$^+$) Calculated for $C_9H_{15}NO_2$: 172.1338. Found [M+H]$^+$: 172.1336.

4.3.7. Preparation of *(S)*-3-(2-(((3-(4-nitrophenyl)prop-2-yn-1-yl)oxy)methyl) pyrrolidin-1-yl) propanenitrile (2.31.1)



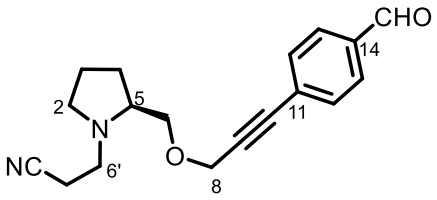
Following general procedure E; Bright yellow oil; yield: 0.28 g, 70%; *v* max /cm^{-1}: 2254 (C≡C) and 1521 (N-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 2H, *J* = 8.5 Hz, Ar), 7.60 (d, 2H, *J* = 8.5 Hz, Ar), 4.43 (s, 2H, C**8**-CH_2), 3.57 (d, 2H, *J* = 5.0 Hz, C**6**-CH_2), 3.24 (m, 2H, C**6'**- CH_2), 2.76 (m, 2H, C**5**-H and C**2**-H), 2.56 (app. t, 2H, *J* = 6.5 Hz, C**3'**-CH_2), 2.38 (m, 1H, C**4**-H), 1.93 (m, 2H, C**3**-CH_2), 1.82 (m, 2H), 1.66 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.3 (C**14**), 132.5 (Ar), 129.4 (C**11**), 123.6 (Ar), 118.4 (CN), 102.3 (C**9**), 90.5 (C**10**), 76.5 (C**14**), 73.9 (C**6**), 59.2 (C**5**), 54.2 (C**8**), 50.7 (C**2**), 28.2 (C**2'**), 23.2 (C**4**), 17.7 (C**3**); HRMS: (CI$^+$) Calculated for $C_{17}H_{20}N_3O_3$: 314.1505. Found [M+H]$^+$: 314.1506.

4.3.8. Preparation of methyl *(S)*-4-(3-((1-(2-cyanoethyl)pyrrolidin-2-yl)methoxy) prop-1-yn-1-yl)benzoate (2.31.2)



Following general procedure E; Yellow oil; yield: 0.32 g, 95%; *v* max /cm^{-1}: 2952 and 2846 (C-H), 2247 (C≡C) and 1717 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.99 (d, 2H, *J* = 8.5 Hz, Ar), 7.50 (d, 2H, *J* = 8.5 Hz, Ar), 4.41 (s, 2H, C**8**-CH_2), 3.92 (s, 3H, C**17**-CH_3), 3.55 (m, 2H, C**6**-CH_2), 3.23 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.75 (m, 2H, C**5**-H and C**6'**-H), 2.53 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (q, 1H, *J* = 7.5 Hz, C**2**-H), 1.94 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.64 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5 (C**15**), 131.7 (Ar), 129.8 (C**14**), 129.5 (Ar), 127.3 (C**11**), 119.1 (CN), 88.2 (C**9**), 85.6 (C**10**), 73.8 (C**6**), 63.0 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 52.2 (C**17**), 50.7 (C**6'**), 28.29 (C**4**), 23.19 (C**3**), 17.74 (C**7'**); HRMS: (CI^+) Calculated for $C_{19}H_{24}N_2O_3$: 328.1787. Found $[M+H]^+$: 328.1783.

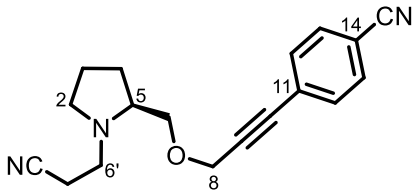
4.3.9. Preparation of *(S)*-3-(2-(((3-(4-formylphenyl)prop-2-yn-1-yl)oxy)methyl) pyrrolidin-1-yl)propanenitrile (2.31.3)



Following general procedure E; Yellow oil; yield 0.16 g, 52%; *v* max /cm^{-1}: 2953 and 2848 (C-H) and 2247 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 10.01 (s, 1H, CHO), 7.83 (d, 2H, *J* = 8.5 Hz, Ar), 7.60 (d, 2H, *J* = 8.5 Hz, Ar), 4.42 (s, 2H, C**8**-CH_2), 3.55 (m, 2H, C**6**-CH_2), 3.23 (m, 1H, - C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.75 (m, 2H, C**5**-H and C**6'**-H), 2.54 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (q, 1H, *J* = 8.5 Hz, C**2**-H), 1.93 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.64 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.4 (C=O), 135.7 (Ar), 132.3 (Ar), 129.5 (Ar), 128.9 (Ar), 119.1 (CN),

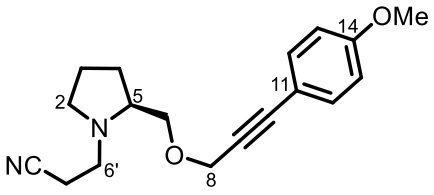
89.3 (C**9**), 85.4 (C**10**), 73.9 (C**6**), 63.0 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.2 (C**3**), 17.8 (C**7'**); HRMS: (CI^+) Calculated for $C_{18}H_{21}N_2O_2$: 297.1603. Found $[M+H]^+$: 297.1599.

4.3.10. Preparation of *(S)*-4-(3-((1-(2-cyanoethyl)pyrrolidin-2-yl)methoxy)prop-1-yn-1-yl)benzonitrile (2.31.4)



Following general procedure E; Yellow oil; yield: 0.26 g, 68%; *v* max /cm^{-1}: 2951 and 2904 and 2854 (C-H) and 2228 (C≡N); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.61 (d, 2H, *J* = 8.5 Hz, Ar), 7.53 (d, 2H, *J* = 8.5 Hz, Ar), 4.41 (s, 2H, C**8**-CH_2), 3.52 (d, 2H, *J* = 5.5 Hz, C**6**-CH_2), 3.24 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.74 (m, 2H, C**6'**-H and C**5**-H), 2.53 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.33 (q, 1H, *J* = 8.0 Hz, C**2**-H), 1.93 (m, 1H C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.63 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.3 (Ar), 132.0 (Ar), 127.5 (C**11**), 119.1 (CN), 118.4 (CN), 111.9 (C**14**), 89.7 (C**9**), 84.7 (C**10**), 74.0 (C**6**), 63.0 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.2 (C**3**), 17.8 (C**7'**); HRMS: (CI^+) Calculated for $C_{18}H_{20}N_3O$: 294.1606. Found $[M+H]^+$: 294.1604.

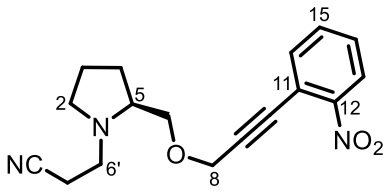
4.3.11. Preparation of *(S)*-3-(2-(((3-(4-methoxyphenyl)prop-2-yn-1-yl)oxy)methyl)pyrrolidin-1-yl)propanenitrile (2.31.5)



Following general procedure E; Pale yellow oil; yield: 0.28 g, 72%; *v* max /cm^{-1}: 2954 and 2905 and 2839 (C-H) and 1606 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.37 (d, 2H, *J* = 8.5 Hz, Ar), 6.83 (d, 2H, *J* = 8.5 Hz, Ar), 4.37 (s, 2H, C**8**-CH_2), 3.81 (s, 3H, -OCH_3), 3.53 (m, 2H, C**6**-CH_2), 3.23 (m, 1H, C**6'**-H), 3.16 (m, 1H, C**2**-H), 2.74

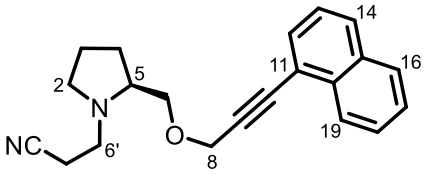
(m, 2H, C**6'**-H and C**5**-H), 2.53 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (q, 1H, *J* = 8.5 Hz, C**2**-H), 1.92 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.63 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.8 (C**14**) 133.3 (Ar), 119.2 (CN), 114.7 (C**11**), 114.0 (Ar), 86.3 (C**9**) , 83.7 (C**10**), 73.5 (C**6**), 63.1 (C**5**), 59.3 (C**8**), 55.3 (OCH_3), 54.4 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.2 (C**3**), 17.7 (C**7'**); HRMS: (CI^+) Calculated for $C_{18}H_{23}N_2O_2$: 299.1760. Found $[M+H]^+$: 299.1762.

4.3.12. *(S)*-3-(2-(((3-(2-nitrophenyl)prop-2-yn-1-yl)oxy)methyl)pyrrolidin-1-yl)propanenitrile (2.31.6)



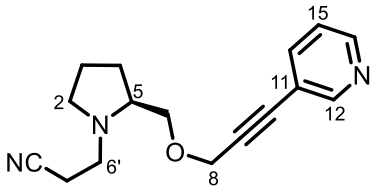
Following general procedure E; Bright yellow oil; yield: 0.23 g, 73%; *ν* max /cm^{-1}: 2932 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 8.04 (d, 1H, *J* = 8.0 Hz, Ar), 7.64 (d, 1H, *J* = 8.0 Hz, Ar), 7.58 (t, 1H, *J* = 7.0 Hz, Ar), 7.47 (t, 1H, *J* = 8.5 Hz, Ar), 4.46 (s, 2H, C**8**-CH_2), 3.60 (m, 2H, C**6**-CH_2), 3.25 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.76 (m, 2H, C**6'**-H and C**5**-H), 2.55 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (q, 1H, *J* = 9.0 Hz, C**2**-H), 1.93 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.67 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1 (C**12**), 134.8 (Ar), 132.8 (Ar), 128.9 (Ar), 124.6 (Ar), 119.2 (CN), 118.0 (C**11**), 93.5 (C**9**), 81.6 (C**10**), 73.5 (C**6**), 63.1 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 50.6 (C**6'**), 28.2 (C**4**), 23.2 (C**3**), 17.7 (C**7'**); HRMS: (CI^+) Calculated for $C_{17}H_{20}N_3O_3$: 314.1505. Found $[M+H]^+$: 314.1505.

4.3.13. Preparation of *(S)*-3-(2-(((3-(naphthalen-1-yl)prop-2-yn-1-yl)oxy)methyl) pyrrolidin-1-yl)propanenitrile (2.31.7)



Following general procedure E; Pale yellow oil; yield: 0.31 g, 99%; *ν* max /cm^{-1}: 3058 (ArC-H), 2950 and 2850 (C-H) and 2246 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 8.32 (d, 1H, *J* = 8.0 Hz, Ar), 7.84 (t, 2H, *J* = 7.0 Hz, Ar), 7.69 (d, 1H, *J* = 7.0 Hz, Ar), 7.55 (m, 2H, Ar), 7.43 (t, 1H, *J* = 7.5 Hz, Ar), 4.55 (s, 2H, C**8**-CH_2), 3.64 (m, 2H, C**6**-CH_2), 3.26 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.78 (m, 2H, C**5**-H and C**6'**-H), 2.54 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (m, 1H, C**2**-H), 1.94 (m, 1H, C**4**-H), 1.78 (m, 2H, C**3**-CH_2), 1.66 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.3 (Ar), 133.2 (Ar), 130.7 (Ar), 129.0 (Ar), 128.3 (Ar), 126.8 (Ar), 126.4 (Ar), 126.1 (Ar), 125.2 (Ar), 120.3 (Ar), 119.2 (CN), 90.0 (C**9**), 84.4 (C**10**), 73.6 (C**6**), 63.1 (C**5**), 59.5 (C**8**), 54.5 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.2 (C**3**), 17.7 (C**7'**); HRMS: (CI$^+$) Calculated for $C_{21}H_{23}N_2O$: 319.1810. Found [M+H]$^+$: 319.1815.

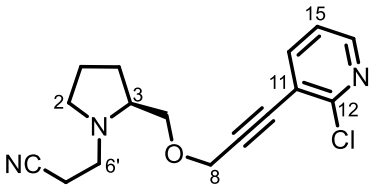
4.3.14. Preparation of *(S)*-3-(2-(((3-(pyridin-3-yl)prop-2-yn-1-yl)oxy) methyl)pyrrolidin-1-yl)propanenitrile (2.31.8)



Following general procedure E; Pale yellow oil; yield: 0.21 g, 83%; *ν* max /cm^{-1}: 3054 and 2981 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 8.68 (s, 1H, Ar), 8.54 (dd, 1H, *J* = 5.0 Hz and 1.5 Hz, Ar), 7.74 (m, 1H, Ar), 7.25 (m, 1H, Ar), 4.41 (s, 2H, C**8**-CH_2), 3.54 (m, 2H, C**6**-CH_2), 3.23 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.75 (m, 2H, C**5**-H and C**6'**-H), 2.53 (t, 2H, *J* = 7.0 Hz, C**7'**-CH_2), 2.34 (m, 1H, C**2**-H), 1.93 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.64 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.5

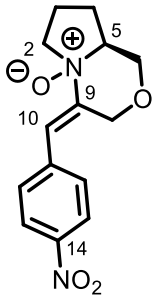
(Ar), 148.9 (Ar), 138.7 (Ar), 123.0 (Ar), 119.8 (CN), 119.1 (Ar), 88.6 (C**9**), 83.0 (Ar), 73.9 (C**6**), 63.0 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.21 (C**3**), 17.76 (C**7'**); HRMS: (CI^+) Calculated for $C_{16}H_{20}N_3O$: 270.1606. Found $[M+H]^+$: 270.1608.

4.3.15. Preparation of *(S)*-3-(2-(((3-(2-chloropyridin-3-yl)prop-2-yn-1-yl)oxy) methyl)pyrrolidin-1-yl)propanenitrile (2.31.8)



Following general procedure E; Yellow oil; yield: 0.26 g, 90%; *v* max /cm^{-1}: 3054 and 2986 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 8.34 (m, 1H, Ar), 7.80 (m, 1H, Ar), 7.21 (m, 1H, Ar), 4.45 (s, 2H, C**8**-CH_2), 3.58 (m, 2H, C**6**-CH_2), 3.23 (m, 1H, C**6'**-H), 3.17 (m, 1H, C**2**-H), 2.76 (m, 2H, C**5**-H and C**6'**-H), 2.53 (t, 2H, *J* = 7.5 Hz, C**7'**-CH_2), 2.34 (m, 1H, C**2**-H), 1.92 (m, 1H, C**4**-H), 1.79 (m, 2H, C**3**-CH_2), 1.65 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.4 (C**12**), 148.5 (C**14**), 141.6 (Ar), 121.9 (Ar), 119.9 (CN), 119.1 (Ar), 93.1 (C**9**), 81.3 (C**10**), 73.7 (C**6**), 63.0 (C**5**), 59.2 (C**8**), 54.4 (C**2**), 50.7 (C**6'**), 28.3 (C**4**), 23.2 (C**3**), 17.8 (C**7'**); HRMS: (CI^+) Calculated for $C_{16}H_{19}N_3OCl$: 304.1217. Found $[M+H]^+$: 304.1222.

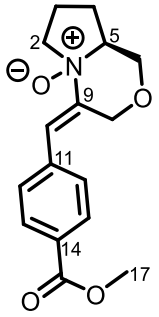
4.3.16. Preparation of (8a*S*)-4-((*E*)-4-nitrobenzylidene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.1)



Following general procedure F; Bright yellow viscous oil; yield: 0.23 g, 86%; *v* max /cm^{-1}: 2993 (C-H), 1518 and 1329 (N-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (m, 2H, C**10**-H and 2 x Ar-H), 7.40 (d, 2H, *J* = 8.5 Hz, 2 x Ar-H), 4.82 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.27 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.10 (m, 1H, C**2**-H), 4.02 (m, 1H, C**6**-H),

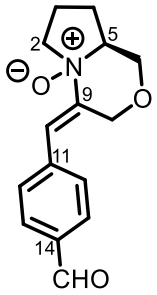
3.90 (m, 1H, C**6**-H), 3.48 (m, 1H, C**5**-H), 3.42 (m, 1H, C**2**-H), 2.76 (m, 1H, C**3**-H), 2.68 (m, 1H, C**3**-H), 2.11 (m, 2H, C**4**-H), 1.73 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.6 (Ar), 138.5 (Ar), 135.9 (Ar), 129.9 (C**10)**, 124.9 (Ar), 123.9 (C**9**), 79.2 (C**5**), 68.2 (C**6**), 66.1 (C**2**), 63.6 (C**8**), 24.8 (C**4**), 20.9 (C**3**); HRMS: (CI^+) Calculated for $C_{14}H_{17}N_2O_4$: 277.1188. Found $[M+H]^+$: 277.1189.

4.3.17. Preparation of (8a*S*)-4-((*E*)-4-(methoxycarbonyl)benzylidene) hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.2)



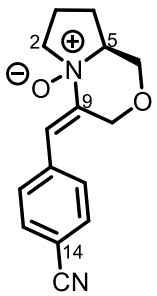
Following general procedure F; Yellow viscous oil; yield: 0.24 g, 83%; *v* max /cm^{-1}: 2955 (C-H) and 1714 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H, C**10**-H), 8.05 (d, 2H, *J* = 8.0 Hz, Ar), 7.29 (d, 2H, *J* = 8.0 Hz, Ar), 4.87 (d, 1H, *J* = 14.5 Hz, C**8**-H), 4.27 (d, 1H, *J* = 14.5 Hz, C**8**-H), 4.09 (m, 1H, C**2**-H), 4.01 (dd, 1H, *J* = 13.0 and 5.0 Hz, C**6**-H), 3.93 (s, 3H, C**17**-CH_3), 3.86 (m, 1H, C**5**-H), 3.47 (m, 1H, C**6**-H), 3.40 (m, 1H, C**2**-H), 2.75 (m, 1H, C**4**-H), 2.64 (m, 1H, C**3**-H), 2.10 (m, 1H, C**3**-H), 1.72 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6 (C**15**), 144.3 (C**11**), 138.4 (C**9**), 129.8 (Ar), 129.7 (**14**), 129.1 (Ar), 125.6 (C**10**), 78.9 (C**5**), 68.0 (C**6**), 66.2 (C**2**), 62.8 (C**8**), 52.2 (C**17**), 24.8 (C**4**), 21.0 (C**3**); HRMS: (CI^+) Calculated for $C_{16}H_{20}NO_4$: 290.1392. Found $[M+H]^+$: 290.1390.

4.3.18. Preparation of (8a*S*)-4-((*E*)-4-formylbenzylidene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.3)



Following general procedure F; Yellow viscous oil; yield: 0.11 g, 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (s, 1H, C**H**O), 8.24 (s, 1H, C**10**-H), 7.91 (d, 2H, *J* = 8.5 Hz, Ar), 7.39 (d, 2H, *J* = 8.5 Hz, Ar), 4.87 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.28 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.09 (m, 1H, C**2**-H), 4.01 (dd, 1H, *J* = 5.0 and 13.0 Hz, C**6**-H), 3.90 (m, 1H, C**5**-H), 3.46 (m, 2H, C**6**-H and C**2**-H), 2.74 (m, 1H, C**4**-H), 2.65 (m, 1H, C**3**-H), 2.11 (m, 1H, C**3**-H), 1.73 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5 (C=O), 139.9 (Ar), 135.8 (Ar), 130.6 (C**10**), 129.9 (Ar), 129.8 (Ar), 125.6 (C**9**), 79.0 (C**5**), 71.9 (C**6**), 68.0 (C**2**), 62.8 (C**8**), 24.8 (C**4**), 21.0 (C**3**); HRMS: (CI^+) Calculated for $C_{15}H_{17}NO_3Na$: 282.1106. Found $[M+Na]^+$: 282.1109.

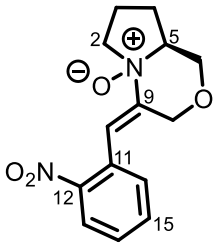
4.3.19. Preparation of (8a*S*)-4-((*E*)-4-cyanobenzylidene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.4)



Following general procedure F; Pale yellow oil; yield: 0.24 g, 88%; *v* max /cm^{-1}: 3052 (alkene C-H), 2980 and 2925 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H, C**10**-H), 7.68 (d, 2H, *J* = 8.0 Hz, 2 x C**13**-H), 7.33 (d, 2H, *J* = 8.0 Hz, 2 x C**12**-H), 4.81 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.25 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.07 (m, 1H, C**2**-H), 4.00 (dd, 1H, *J* = 13.0 and 5.0 Hz, C**6**-H), 3.90 (m, 1H, C**5**-H), 3.46 (m, 2H, C**6**-H and C**2**-H), 2.74 (m, 1H, C**4**-H), 2.65 (m, 1H, C**3**-H), 2.11 (m, 1H, C**3**-H), 1.72 (m, 1H, C**4**-

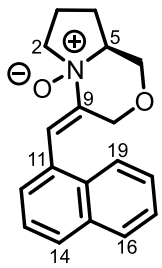
H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.0 (C**11**), 138.5 (C**9**), 132.4 (Ar), 129.8 (Ar), 125.2 (C**10**), 118.4 (CN), 112.0 (C**14**), 79.0 (C**5**), 68.1 (C**6**), 66.1 (C**2**), 62.64 (C**8**), 24.76 (C**4**), 21.02 (C**3**); HRMS: (CI^+) Calculated for $C_{15}H_{16}N_2O_2Na$: 279.1109. Found $[M+Na]^+$: 289.1112.

4.3.20. (8a*S*)-4-((*E*)-2-nitrobenzylidene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.5)



Following general procedure F; Bright yellow viscous oil; yield: 0.18 g, 84%; *v* max /cm^{-1}: 2980 (C-H), 1521 and 1343 (N-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (dd, 1H, *J* = 1.0 and 8.0 Hz, C**13**-H) 7.90 (s, 1H, C**10**-H), 7.67 (t, 1H, *J* = 8.0 Hz, Ar), 7.54 (t, 1H, *J* = 8.0 Hz, Ar), 7.36 (d, 1H, *J* = 7.5 Hz, Ar), 4.62 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.20 (dd, 1H, *J* = 13.0 and 4.0 Hz, C**6**-H), 4.15 (m, 1H, C**2**-H), 4.09 (d, 1H, *J* = 13.5 Hz, C**8**-H), 3.80 (m, 1H, C**5**-H), 3.57 (m, 2H, C**6**-H and C**2**-H), 2.52 (m, 2H, C**4**-H and C**3**-H), 2.20 (m, 1H, C**3**-H), 1.91 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.8 (C**12**), 131.8 (C**15**), 129.6 (C**10**), 129.4 (Ar), 125.2 (C**9**), 121.3 (C**13**), 78.1 (C**5**), 66.2 (C**6**), 65.9 (C**2**), 62.4 (C**8**), 23.6 (C**4**), 19.7 (C**3**); HRMS: (CI^+) Calculated for $C_{14}H_{16}N_2O_4Na$: 299.1007. Found $[M+Na]^+$: 299.1009.

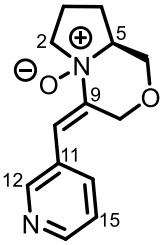
4.3.21. Preparation of (8a*S*,*E*)-4-(naphthalen-1-ylmethylene) hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.6)



Following general procedure F; Colourless oil; yield: 0.08 g, 57%; *v* max /cm^{-1}: 2963 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H, C**10**-H), 7.99 (m, 1H, Ar), 7.85 (m, Ar), 7.52 (m, 2H, Ar), 7.45 (t, 1H, Ar), 7.21 (d, 1H, Ar), 4.72 (d, 1H, C**8**-H), 4.18 (m,

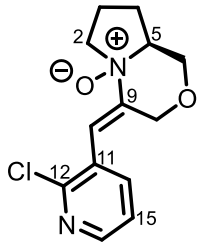
2H, C**8**-H, C**2**-H), 4.01 (dd, 1H, C**6**-H), 3.92 (m, 1H, C**5**-H), 3.49 (m, 2H, C**2**-H and C**6**-H), 2.73 (m, 1H, C**4**-H), 2.64 (m, 1H, C**3**-H), 2.11 (m, 1H, C**3**-H), 1.74 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.8 (Ar), 133.4 (Ar), 131.7 (Ar), 130.9 (Ar), 128.8 (Ar), 128.4 (C**10**), 126.6 (Ar), 126.6 (Ar), 126.3 (Ar), 125.1 (Ar), 124.9 (C**7**), 124.5 (Ar), 78.6 (C**5**), 67.7 (C**6**), 65.8 (C**2**), 63.0 (C**8**), 24.4 (C**4**), 20.8 (C**3**); HRMS: (CI^+) Calculated for $C_{18}H_{20}NO_2$: 282.1494. Found $[M+H]^+$: 282.1490.

4.3.22. Preparation of (8a*S*,*E*)-4-(pyridin-3-ylmethylene)hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.7)



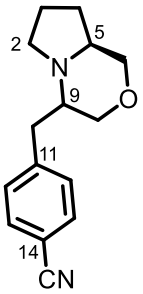
Following general procedure F; Pale yellow oil; yield: 0.16 g, 47%; *v* max /cm^{-1}: 3047 (alkene C-H), 2924 and 2855 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, 1H, Ar), 8.45 (s, 1H, Ar), 8.18 (s, 1H, C**10**-H), 7.59 (d, 1H, Ar), 7.33 (m, 1H, Ar), 4.85 (d, 1H, C**8**-H), 4.29 (d, 1H, C**8**-H), 4.09 (m, 1H, C**2**-H), 4.00 (dd, 1H, C**6**-H), 3.88 (m, 1H, C**5**-H), 3.45 (m, 2H, C**6**-H and C**2**-H), 2.75 (m, 1H, C**4**-H), 2.65 (m, 1H, C**3**-H), 2.09 (m, 1H, C**3**-H), 1.71 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.6 (Ar), 149.3 (Ar), 144.6 (Ar), 136.5 (C**9**), 129.7 (C**10**), 123.5 (Ar), 123.1 (Ar), 79.0 (C**5**), 68.1 (C**6**), 66.3 (C**2**), 62.7 (C**8**), 24.8 (C**4**), 21.0 (C**3**); HRMS: (CI^+) Calculated for $C_{13}H_{16}N_2O_2Na$: 255.1109. Found $[M+Na]^+$: 255.1113.

4.3.23. Preparation of (8a*S*,*E*)-4-((2-chloropyridin-3-yl)methylene) hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide (2.34.8)



Following general procedure F; Brown oil; yield: 0.08 g, 50%; *v* max /cm^{-1}: 3054 (C-H) and 2980 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, 1H, *J* = 4.5 Hz, Ar), 7.86 (s, 1H, C**10**), 7.57 (d, 1H, *J* = 7.5 Hz, Ar), 7.30 (m, 1H, Ar), 4.61 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.19 (d, 1H, *J* = 14.0 Hz, C**8**-H), 4.13 (m, 2H, C**2**-H and C**6**-H), 3.84 (m, 1H, C**5**-H), 3.52 (m, 2H, C**2**-H and C**6**-H), 2.60 (m, 2H, C**3**-H and C**4**-H), 2.15 (m, 1H, C**3**-H), 1.83 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.7 (C**12**), 149.4 (C**14**), 145.1 (Ar), 139.1 (C**11**), 129.1 (C**10**), 122.4 (C**9**), 120.7 (Ar), 78.5 (C**5**), 67.1 (C**6**), 66.0 (C**2**), 62.6 (C**8**), 24.0 (C**4**), 20.3 (C**3**); HRMS: (CI$^+$) Calculated for $C_{13}H_{16}N_2O_2Cl$: 267.0900. Found $[M+H]^+$: 267.0902.

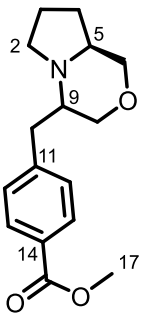
4.3.24. Preparation of 4-(((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)methyl)benzonitrile (2.38.1)



Following general procedure G; Dark yellow oil; 0.03 g; 6%; *v* max /cm^{-1}: 2961 and 2850 and 2802 (C-H) and 2227 (C≡N); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.58 (d, 2H, *J* = 8.0 Hz, Ar), 7.29 (d, 2H, *J* = 8.0 Hz, Ar), 3.95 (dd, 1H, *J* = 3.0 and 11.0 Hz, C**6**-H), 3.53 (dd, 1H, *J* = 2.0 and 11.0 Hz, C**8**-H), 3.33 (td, 1H, *J* = 2.0 and 9.5 Hz, C**2**-H), 3.26 (t, 1H, *J* = 10.5 Hz, C**6**-H), 3.15 (t, 1H, *J* = 9.5 Hz, C**8**-H), 3.07 (app. d, 1H, *J* = 9.0 Hz, C**10**-H), 2.58 (m, 2H, C**9**-H and C**10**-H), 2.21 (m, 1H, C**5**-H), 2.11 (q, 1H, *J* = 8.5 Hz, C**2**-H), 1.79 (m, 3H, C**3**-CH_2 and C**4**-H), 1.38 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.0 (C**11**), 132.2 (Ar), 129.9 (Ar), 118.8 (CN), 110.4 (C**14**),

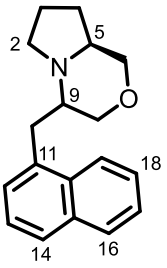
71.3 (C**6**), 69.9 (C**8**), 63.2 (C**9**), 62.7 (C**5**), 51.2 (C**2**), 37.6 (C**10**), 25.8 (C**4**), 20.4 (C**3**); HRMS: (CI^+) Calculated for $C_{15}H_{19}N_2O$: 243.1497. Found $[M+H]^+$: 243.1492.

4.3.25. Preparation of methyl 4-(((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)methyl)benzoate (2.38.2)



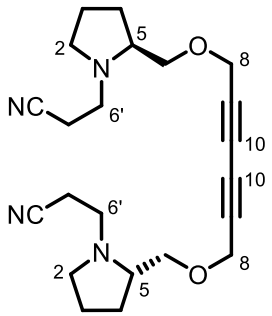
Following general procedure G; Yellow oil; yield: 0.18 g, 58%; *v* max /cm^{-1}: 2953 and 2845 and 2799 (C-H) and 1717 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 7.95 (d, 2H, *J* = 8.0 Hz, Ar), 7.24 (d, 2H, *J* = 8.0 Hz, Ar), 3.95 (dd, 1H, *J* = 3.0 and 11.0 Hz, C**6**-H), 3.90 (s, 3H, -OCH_3), 3.55 (dd, 1H, *J* = 2.5 and 11.0 Hz, C**8**-H), 3.33 (t, 1H, *J* = 8.5 Hz, C**2**-H), 3.26 (app. t, 1H, *J* = 10.5 Hz, C**6**-H), 3.15 (app. t, 1H, *J* = 11.0 Hz, C**8**-H), 3.08 (dd, 1H, *J* = 3.0 and 12.5 Hz, C**10**-H), 2.56 (m, 2H, C**9**-H and C**10**-H), 2.19 (m, 1H, C**5**-H), 2.11 (m, 1H, C**2**-H), 1.78 (m, 3H, C**3**-CH_2 and C**4**-H), 1.38 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0 (C=O), 143.8 (Ar), 129.8 (Ar), 129.1 (Ar), 128.4 (Ar), 71.4 (C**6**), 70.18 (C**8**), 63.4 (C**9**), 62.8 (C**5**), 52.0 (C**17**), 51.2 (C**2**), 37.6 (C**10**), 25.8 (C**4**), 20.5 (C**2**); HRMS: (CI^+) Calculated for $C_{16}H_{22}NO_3$: 276.1600. Found $[M+H]^+$: 276.599.

4.3.26. Preparation of (8a*S*)-4-(naphthalen-1-ylmethyl)hexahydro-1H-pyrrolo[2,1-c][1,4]oxazine (2.38.3)



Following general procedure G; Brown oil; 0.22 g, 86%; *v* max /cm^{-1}: 2960 and 2846 and 2800 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 8.04 (d, 1H, *J* = 8.0 Hz, Ar), 7.85 (d, 1H, *J* = 9.0 Hz, Ar), 7.74 (d, 1H, *J* = 8.0 Hz, Ar), 7.50 (m, 2H, Ar), 7.38 (t, 1H, *J* = 7.0 Hz, Ar), 7.30 (d, 1H, *J* = 7.0 Hz, Ar), 3.94 (dd, 1H, *J* = 3.0 and 7.0 Hz, C**6**-H), 3.61 (m, 2H, C**10**-H and C**2**-H), 3.49 (dd, 1H, *J* = 3.0 and 11.0 Hz, C**8**-H), 3.29 (m, 2H, C**6**-H and C**8**-H), 2.87 (m, 1H, C**10**-H), 2.76 (m, 1H, C**9**-H), 2.34 (m, 1H, C**2**-H), 2.21 (m, 1H, C**5**-H), 1.85 (m, 3H, C**3**-CH_2 and C**4**-H), 1.41 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.2 (Ar), 134.0 (Ar), 132.0 (Ar), 128.9 (Ar), 127.5 (Ar), 127.3 (Ar), 125.9 (Ar), 125.6 (Ar), 125.4 (Ar), 123.9 (Ar), 71.5 (C**6**), 71.3 (C**8**), 70.7 (C**9**), 62.8 (C**5**), 51.1 (C**2**), 34.5 (C**10**), 25.8 (C**4**), 20.6 (C**3**); HRMS: (CI$^+$) Calculated for $C_{18}H_{22}NO$: 268.1701. Found [M+H]$^+$: 168.1703.

4.3.27. Preparation of 3,3'-((*2S,2'S*)-((hexa-2,4-diyne-1,6-diylbis(oxy)) bis(methylene))bis(pyrrolidine-2,1-diyl))dipropanenitrile (2.39)

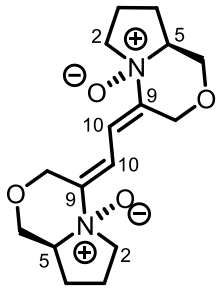


To a stirred solution of *(S)*-3-(2-((prop-2-yn-1-yloxy)methyl)pyrrolidin-1-yl)propanenitrile **2.19** (0.20 g, 1.04 mmol, 1.0 eq.) in DCM (10 mL) was added TMEDA (0.06 mL, 0.42 mmol, 0.25eq.) and CuI (0.04 g, 0.21 mmol, 0.2 eq.). To reaction mixture was allowed to stir under an oxygen atmosphere for 24 hours. A colour change, from colourless, to green, to blue was observed in this time. Solvents

were removed *in vacuo*. The residue was taken up in H_2O (20 mL) and EtOAc (20 mL) was extracted with ethyl acetate (3 x 20 mL) and the combined organic extracts were dried ($MgSO_4$). Solvent was then removed to give the crude product. The crude material was purified by FCC (eluting with 4:1 EtOAc:hexanes) to afford the title compound (0.10 g, 52%) as a yellow oil.

v max /cm^{-1}: 3054 and 2982 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ 4.27 (m, 4H, 2 x C**8**-CH_2), 3.50 (m, 4H, 2 x C**6**-CH_2), 3.20 (m, 4H, 2 x C**2**-H and 2 x C**6'**-H), 2.74 (m, 4H, 2 x C**5**-H and 2 x C**6'**-H), 2.54 (t, 4H, *J* = 7.0 Hz, 2 x C**7'**-CH_2), 2.35 (q, 2H, *J* = 8.5 Hz, 2 x C**2**-H), 1.92 (m, 2H, 2 x C**4**-H), 1.80 (m, 4H, 2 x C**3**-CH_2), 1.62 (m, 2H, 2 x C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 119.1 (2 x CN), 75.5 (2 x C**6**), 73.8 (2 x C**10**), 70.4 (2 x C**9**), 63.0 (2 x C**5**), 59.0 (2 x C**8**), 54.4 (2 x C**2**), 50.7 (2 x C**6'**), 28.2 (2 x C**4**), 23.2 (2 x C**3**), 17.7 (2 x C**7'**); HRMS: (CI^+) Calculated for $C_{22}H_{30}N_4O_2Na$: 425.2266. Found $[M+Na]^+$: 425.2271.

4.3.28. Preparation of (4*E*,4'*E*,5*R*,5'*R*,8a*S*,8a'*S*)-4,4'-(ethane-1,2-diylidene) bis(hexahydropyrrolo[2,1-c][1,4]oxazine 5(1H)-oxide) (2.41)

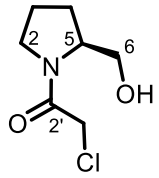


To a stirred solution of 3,3'-((2S,2'S)-((hexa-2,4-diyne-1,6-diylbis(oxy))bis(methylene))bis(pyrrolidine-2,1-diyl))dipropanenitrile **2.39** (0.12 g, 0.31 mmol, 1.0 eq.) in DCM (15 mL) at -78 °C was added *m*-CPBA (0.13 g, 0.75 mmol, 2.4 eq.) and K_2CO_3 (0.11 g, 0.76 mmol, 2.5 eq.). The reaction was allowed to warm to ambient temperature slowly overnight. The reaction was then heated to reflux for 2 days. Solvent was then removed to give the crude product. The crude material was purified by FCC (eluting with 1:1 to 0:1 EtOAc:MeOH) to afford the title compound (37 mg, 38%) as a viscous oil.

v max /cm^{-1}: 3054 and 2985 (C-H); ^{1}H NMR (500 MHz, CD_3OD) δ 7.59 (s, 2H, 2 x C**10**-H), 4.97 (d, 2H, *J* = 15.0 Hz, 2 x C**8**-H), 4.38 (d, 2H, *J* = 15.0 Hz, 2 x C**8**-H),

4.25 (m, 2 x 2H, C**2**-H), 4.00 (dd, 2H, *J* = 4.5 and 12.5 Hz, 2 x C**6**-H), 3.79 (m, 2H, 2 x C**5**-H), 3.64 (m, 2H, 2 x C**6**-H), 3.26 (m, 2H, 2 x C**2**-H), 2.57 (m, 2H, 2 x C**4**-H), 2.43 (m, 2H, 2 x C**3**-H), 2.18 (m, 2H, 2 x C**3**-H), 1.78 (m, 2H, 2 x C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.0 (2 x C**9**), 116.1 (2 x C**10**), 79.2 (2 x C**5**), 67.3 (2 x C**6**), 65.6 (2 x C**2**), 61.4 (2 x C**8**), 23.7 (2 x C**4**), 20.2 (2 x C**3**); HRMS: (CI^+) Calculated for $C_{16}H_{24}N_2O_4Na$: 331.1634. Found $[M+Na]^+$: 331.1630.

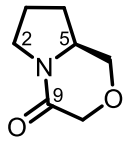
4.3.29. Preparation of *(S)*-2-chloro-1-(2-(hydroxymethyl)pyrrolidin-1-yl)ethan-1-one (2.42)



To a stirred solution of *L*-prolinol (4.00 g, 39.5 mmol, 1.0 eq.) and sodium acetate (6.49 g, 79.1 mmol, 2.0 eq.) in acetone (55 mL) and H_2O (27 mL) at 0-5 °C was added chloroacetyl chloride (4.47 g, 39.5 mmol, 1.0 eq.). The reaction was warmed to ambient temperature and stirred for a further 2 hours. The acetone was then removed *in vacuo* and the remaining aqueous was extracted with chloroform (3 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:19 MeOH:DCM) to afford the title compound (3.82 g, 55%) as a yellow oil.

v max /cm^{-1}: 3050 (br. O-H) and 1611 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 (br. s, 1H, OH), 4.21 (m, 1H, C**6**-H), 4.06 (d, 2H, *J* = 13.0 Hz, C**3'**-CH_2), 3.70 (m, 1H, C**6**-H), 3.62 (m, 2H, C**5**-H and C**2**-H), 3.52 (m, 1H, C**2**-H), 2.05 (m, 2H, C**3**-H and C**4**-H), 1.92 (m, 1H, C**3**-H), 1.69 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 (C**2'**), 66.3 (C**6**), 61.9 (C**5**), 48.1 (C**3'**), 42.4 (C**2**), 28.1 (C**4**), 24.5 (C**3**); HRMS: (CI^+) Calculated for $C_7H_{13}ClNO_2$: 178.0629. Found $[M+H]^+$: 178.0633.

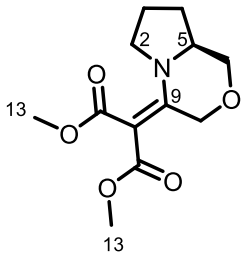
4.3.30. Preparation of *(S)*-tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-one (2.43)



To a stirred solution of alcohol **2.42** (3.82 g, 21.57 mmol, 1.0 eq.) in THF (70 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 1.23 g, 3.85 mmol, 1.3 eq.) in portions. The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was quenched with methanol and solvents were removed *in vacuo*. The residue was taken up in chloroform (50 mL) and washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:49 MeOH:EtOAc) to afford the title compound (1.85 g, 61%) as an off white solid.

v max /cm^{-1}: 1648 (C=O) and 1106 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (d, 1H, *J* = 16.5 Hz, C**8**-H), 4.19 (dd, 1H, *J* = 11.5, 4.0 Hz, C**6**-H), 4.01 (d, 1H, *J* = 16.5 Hz, C**8**-H), 3.71 (m, 2H, C**5**-H and C**2**-H), 3.50 (m, 1H, C**2**-H), 3.24 (m, 1H, C**6**-H), 2.03 (m, 2H, C**4**-H and C**3**-H), 1.84 (m, 1H, C**3**-H), 1.39 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3 (C**9**), 69.1 (C**6**), 66.9 (C**8**), 57.1 (C**5**), 44.6 (C**2**), 28.8 (C**4**), 22.1 (C**3**); HRMS: (CI^+) Calculated for $C_7H_{11}NO_2$: 142.0863. Found $[M+H]^+$: 142.0864.

4.3.31. Preparation of dimethyl *(S)*-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)malonate (2.45.1)

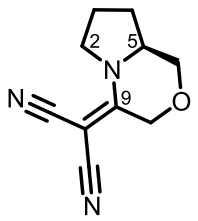


Enolate formation: NaHMDS (1M in THF, 3.12 mL, 3.12 mmol, 2.2 eq.) was added to dimethylmalonate (0.24 mL, 2.13 mmol, 1.5 eq.) in DCM (5 mL) at -78 °C. The reaction mixture was allowed to warm to 0 °C and stirred for a further 1 hour.

To a stirred solution of lactam **2.43** (0.20 g, 1.42 mmol, 1.0 eq.) in DCM (8 mL) at -78 °C was added Tf_2O (0.26 mL, 1.56 mmol, 1.1 eq.). The reaction was warmed to 0 °C and stirred for a further 1 hour. The reaction mixture was the cooled to -78 °C the preformed enolate was added *via* cannula. The reaction was allowed to warm to ambient temperature slowly and stirred overnight. The reaction mixture was diluted with sat.NH_4Cl solution 30 mL and extracted with DCM (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:1 to 1:4 hexanes:EtOAc) to afford the title compound (0.20 g, 56%) as pale yellow oil.

v max /cm^{-1}: 1689 (C=O), 1523 (C=C) and 1113 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78 (m, 2H, C**8**-CH_2), 4.16 (dd, 1H, *J* = 11.0, 4.5 Hz, C**6**-H), 3.70 (s, 6H, 2 x C**13**-CH_3), 3.65 (m, 1H, C**5**-CH_2), 3.47 (m, 1H, C**2**-H), 3.40 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 3.22 (m, 1H, C**2**-H), 2.01 (m, 2H, C**3**-H and C**4**-H), 1.89 (m, 1H, C**4**-H), 1.43 (m, 1H, C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3 (C**11**), 159.0 (C**9**), 90.8 (C**10**), 68.7 (C**6**), 66.2 (C**8**), 56.9 (C**5**), 51.6 (C**13**), 50.3 (C**2**), 27.5 (C**4**), 22.7 (C**3**); HRMS: (CI^+) Calculated for $C_{12}H_{18}NO_5$: 256.1179. Found $[M+H]^+$: 256.1175.

4.3.32. Preparation of *(S)*-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)malononitrile (2.45.2)



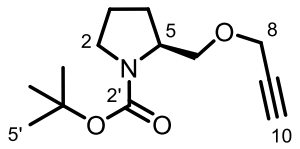
Enolate formation: NaHMDS (1M in THF, 4.68 mL, 4.68 mmol, 2.2 eq.) was added to malononitrile (0.20 mL, 3.19 mmol, 1.5 eq.) in DCM (5 mL) at -78 °C. The reaction mixture was allowed to warm to 0 °C and stirred for a further 1 hour.

To a stirred solution of lactam **2.43** (0.30 g, 2.13 mmol, 1.0 eq.) in DCM (8 mL) at -78 °C was added Tf_2O (0.39 mL, 2.34 mmol, 1.1 eq.). The reaction was warmed to 0 °C and stirred for a further 1 hour. The reaction mixture was the cooled to -78 °C and the preformed enolate was added *via* cannula. The reaction was allowed to warm to ambient temperature slowly and stirred overnight. The reaction mixture was diluted

with sat.NH_4Cl solution 30 mL and extracted with DCM (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:1 to 1:4 hexanes:EtOAc) to afford the title compound (0.22 g, 40%) as a pale yellow oil.

v max /cm^{-1}: 2202 and 2184 (C≡N) and 1580 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (d, 1H, *J* = 17.0 Hz, C**8**-H), 4.37 (d, 1H, *J* = 17.0 Hz, C**8**-H), 4.28 (dd, 1H, *J* = 11.5, 4.0 Hz, C**6**-H), 4.20 (m, 1H, C**2**-H), 3.97 (m, 1H, C**2**-H), 3.64 (m, 1H, C**5**-H), 3.34 (dd, 1H, *J* = 11.0, 10.0 Hz, C**6**-H), 2.21 (m, 1H, C**4**-H), 2.13 (m, 1H, C**3**-H), 2.97 (m, 1H, C**4**-H), 1.41 (m, 1H, C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1 (C**9**), 115.4 (C**11**), 68.4 (C**6**), 64.9 (C**8**), 58.3 (C**5**), 50.4 (C**2**), 46.3 (C**10**), 27.5 (C**4**), 22.6 (C**3**); HRMS: (CI^+) Calculated for $C_{10}H_{12}N_3O$: 190.0975. Found $[M+H]^+$: 190.0972.

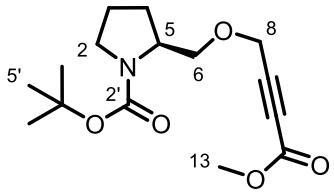
4.3.33. Preparation of *tert*-butyl *(S)*-2-((prop-2-yn-1-yloxy)methyl)pyrrolidine-1-carboxylate (2.51)



NaH (60% dispersion in mineral oil, 2.39 g, 59.63 mmol, 1.2 eq.) was added in portions to a stirred solution of *N*-boc-*L*-prolinol (10.00 g, 49.69 mmol, 1.0 eq.) in THF (150 mL) under a N_2 atmosphere at 0 °C. The reaction mixture was allowed to stir for 0.5 hours at 0 °C followed by dropwise addition of propargyl bromide (80% wt. in toluene, 6.65 mL, 59.63 mmol, 1.1 eq.). The reaction was allowed to warm to ambient temperature and was monitored by TLC. Upon complete disappearance of starting material the reaction was quenched with methanol and solvents were removed *in vacuo* to give a cloudy yellow residue. The residue was taken up in EtOAc (200 mL) and washed with saturated sodium bicarbonate solution (150 mL) and brine (150 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:17 EtOAc:hexanes) to afford the title compound (10.92 g, 95%) as a pale yellow oil.

v max /cm^{-1}: 3271 (C≡C-H), 2975 (C-H), 2117 (C≡C), 1687 (C=O), 1163 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (2H, s, C**8**-CH_2), 3.93 (1H, br m, C**5**-H), 3.65 (1H, dd, *J* = 9.0, 3.5 Hz, C**6**-H), 3.41 (3H, br m, C**6**-H and C**2**-CH_2), 2.41 (1H, s, C**10**-H), 1.84 (4H, br m, C**4**-CH_2 and C**3**-CH_2), 1.47 (9H, s, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.6 (C**2'**), 79.9 (C**4'**), 79.3 (C**9**), 74.2 (C**10**), 70.8 (C**6**), 58.4 (C**5**), 56.3 (C**8**), 46.4 (C**2**), 28.5 (C**5'**), 23.7 (C**4**), 22.9 (C**3**); HRMS: (ES$^+$) Calculated for $C_{13}H_{21}NO_3Na$: 262.1419. Found $[M+Na]^+$: 262.1415.

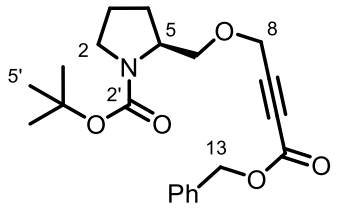
4.3.34. Preparation of *tert*-butyl *(S)*-2-(((4-methoxy-4-oxobut-2-yn-1-yl)oxy)methyl)pyrrolidine-1-carboxylate (2.49.1)



n-BuLi 2.5 M in hexanes (7.58 mL, 18.95 mmol, 1.1 eq.) was added dropwise to a stirred solution of propargyl ether **2.51** (4.0 g, 17.23 mmol, 1.0 eq.) in THF (50 mL) under a N_2 atmosphere at -78 °C. The reaction mixture was allowed to stir for 0.5 hours at -78 °C followed by dropwise addition of methyl chloroformate (1.45 mL, 18.95 mmol, 1.1 eq.). The reaction mixture was allowed to warm to ambient temperature and was monitored by TLC. Upon complete disappearance of starting material the reaction was diluted with sat. NH_4Cl solution (100 mL) and product extracted with EtOAc (3 x 80 mL). The organic phase extracts were combined and washed with brine, dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:19 to 1:3 EtOAc:hexanes) to afford the title compound (4.27 g, 83%) as a pale yellow oil.

v max /cm^{-1}: 1704 and 1677 (C=O) and 2215 (C≡C); ^{1}H NMR (400 MHz, CDCl3) δ 4.28 (br. m, 2H, C**8**-CH_2), 3.93 (br. m, 1H, C**6**-H), 3.77 (s, 3H, C**13**-CH_3), 3.65 (dd, 1H, J = 9.0, 3.5 Hz, C**6**-H), 3.43 (m, 3H, C**2**-CH_2 and C**5**-H), 1.93 (m, 3H, C**4**-CH_2 and C**3**-H), 1.81 (m, 1H, C**3**-H), 1.47 (s, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.4 (C**2'**), 153.6 (C**11**), 83.4 (C**4'**), 79.3 (C**9**), 77.7 (C**10**), 71.2 (C**2**), 70.9 (C**8**), 58.2 (C**5**), 56.2 (C**6**), 52.8 (C**13**), 28.5 (C**5'**), 23.5 (C**4**), 23.0 (C**3**); HRMS: (ES$^+$) Calculated for $C_{15}H_{23}NO_5Na$: 320.1474. Found $[M+Na]^+$: 320.1462.

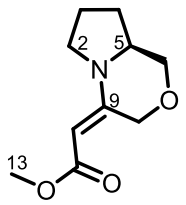
4.3.35. Preparation of *tert*-butyl *(S)*-2-(((4-(benzyloxy)-4-oxobut-2-yn-1-yl)oxy)methyl)pyrrolidine-1-carboxylate (2.49.2)



n-BuLi 2.5 M in hexanes (7.58 mL, 18.95 mmol, 1.1 eq.) was added dropwise to a stirred solution of propargyl ether **2.51** (4.0 g, 17.23 mmol, 1.0 eq.) in THF (50 mL) under a N_2 atmosphere at -78 °C. The reaction mixture was allowed to stir for 0.5 hours at -78 °C followed by dropwise addition of benzyl chloroformate (2.71 mL, 18.95 mmol, 1.1 eq.). The reaction mixture was allowed to warm to ambient temperature and was monitored by TLC. Upon complete disappearance of starting material the reaction was diluted with sat. NH_4Cl solution (100 mL) and product extracted with EtOAc (3 x 80 mL). The organic phase extracts were combined and washed with brine, dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:19 to 1:3 EtOAc:hexanes) to afford the title compound (4.82 g, 75%) as a brown oil.

v max/ cm^{-1} : 1686 and 1648 (C=O), 2239 (C≡C) and 721 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (br. s, 5H, Ar), 5.20 (br. s, 2H, C**13**-CH_2), 4.27 (br. s, 2H, C**8**-CH_2), 3.91 (br. m, 1H, C**6**-H), 3.64 (dd, *J* = 9.0, 3.5 Hz, C**6**-H), 3.32 (m, 3H, C**2**-CH_2 and C**5**-H), 1.85 (m, 3H, C**4**-CH_2 and C**3**-H), 1.79 (m, 1H, C**3**-H), 1.44 (s, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5 (C**2'**), 152.9 (C**11**), 128.7 (Ar), 128.6 (Ar), 128.2 (Ar), 127.7 (Ar), 88.8 (C**4'**), 77.3 (C**9**), 72.9 (C**10**), 71.2 (C**2**), 67.7 (C**8**), 58.2 (C**13**), 56.2 (C**5**), 46.6 (C**6**), 28.5 (C**5'**), 22.9 (C**4**), 20.9 (C**3**); HRMS: (ES^+) Calculated for $C_{21}H_{27}NO_5Na$: 396.1787. Found $[M+Na]^+$: 236.1780.

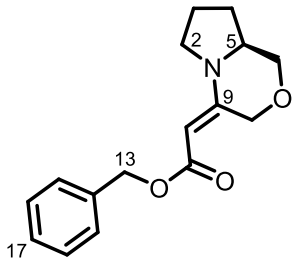
4.3.36. Preparation of methyl (*S*,*E*)-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetate (2.53.1)



TFA (1.30 mL, 16.82 mmol, 10 eq.) was added to a stirred solution of boc protected amine **2.49.1** (0.5 g, 1.68mmol, 1.0 eq.) in DCM (5 mL) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 3 hours. Solvents were removed *in vacuo* and the residue taken up in H_2O (10 mL). The pH was adjusted to pH 12 using 2 M NaOH solution and stirred for a further 0.5 hours and product was extracted with DCM (3 x 10 mL) to afford the title compound (0.32 g, 96%) as a pale yellow oil.

v max/ cm^{-1} :1648 (C=O), 1586 (C=C) and 1175 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.63 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.40 (s, 1H, C**10**-H), 4.17 (dd, 1H, *J* = 11.0, 4.0 Hz, C**6**-H), 3.60 (s, 3H, C**13**-CH_3), 3.53 (m, 1H, C**5**-H), 3.29 (m, 1H, C**6**-H), 3.24 (m, 2H, C**2**-CH_2), 2.10 (m, 1H, C**3**-H), 2.01 (m, 1H, C**4**-H), 1.87 (m, 1H, C**3**-H), 1.34 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.6 (C**11**), 157.4 (C**9**), 79.4 (C**10**), 69.1 (C**6**), 65.7 (C**8**), 56.4 (C**5**), 50.0 (C**13**), 47.8 (C**2**), 28.7 (C**4**), 22.5 (C**3**); HRMS: (CI^+) Calculated for $C_{10}H_{16}NO_3$: 198.1130. Found $[M+H]^+$: 198.1127.

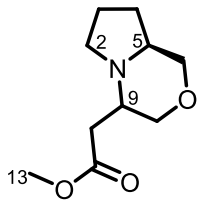
4.3.37. Preparation of benzyl (*S*,*E*)-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetate (2.53.2)



TFA (3.10 mL, 40.19 mmol, 10 eq.) was added to a stirred solution of boc protected amine **2.49.2** (1.5 g, 4.02 mmol, 1.0 eq.) in DCM (15 mL) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 3 hours. Solvents were removed *in vacuo* and the residue taken up in H_2O (30 mL). The pH was adjusted to pH 12 using 2 M NaOH solution and stirred for a further 0.5 hours and product was extracted with DCM (3 x 30 mL) to afford the title compound (0.84 g, 76%) as a brown oil.

v max/ cm^{-1} : 1656 (C=O) and 692 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (m, 5H, Ar), 5.28 (d, 1H, *J* = 17.5 Hz, C**8**-H), 5.14 – 5.05 (m, 2H, C**13**-CH_2), 4.67 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.49 (s, 1H, C**10**-H), 4.19 (dd, 1H, *J* = 11.0, 4.0 Hz, C**5**-H), 3.55 (m, 1H, C**5**-H), 3.33 (m, 2H, C**2**-CH_2), 3.26 (m, 1H, C**6**-H), 2.10 (m, 1H, C**3**-H), 2.03 (m, 1H, C**4**-H), 1.89 (m, 1H, C**3**-H), 1.35 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9 (C**11**), 157.7 (C**9**), 137.7 (C**14**), 128.4 (Ar), 128.0 (Ar), 127.7 (C**17**), 79.6 (C**10**), 69.0 (C**6**), 65.8 (C**8**), 64.4 (C**13**), 56.4 (C**5**), 47.9 (C**2**), 28.7 (C**3**), 22.5 (C**4**); HRMS: (CI^+) Calculated for $C_{16}H_{20}NO_3$: 274.1443. Found $[M+H]^+$: 274.1440.

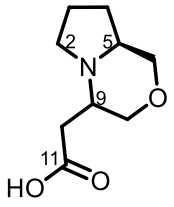
4.3.38. Preparation of methyl 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetate (2.53.3)



To a solution of benzyl (S,E)-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetate **2.53.1** (1.00 g, 5.07 mmol, 1.0 eq.) in MeOH (50 mL) was added 10% Pd/C (0.27 g, 0.25 mmol, 0.05 eq.), and the reaction mixture was allowed to stir for 18 h at rt under a H_2 atmosphere. The reaction was filtered through a Celite pad, and solvents were removed *in vacuo* to give the pure title compound (1.01 g, quant.) as a pale yellow oil.

v max/ cm^{-1} : 1724 (C=O) and 1116 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.98 (dd, 1H, *J* = 10.5, 3.0 Hz, C**6**-H), 3.88 (dd, 1H, *J* = 11.0, 3.0 Hz, C**8**-H), 3.71 (s, 3H, C**13**-CH_3), 3.26 (app. t, 1H, *J* = 10.5 Hz, C**6**-H), 3.19 (m, 2H, C**8**-H and C**2**-H), 2.79 (m, 1H, C**9**-H), 2.61 (dd, 1H, *J* = 15.5, 5.0 Hz, C**10**-H), 2.23 (m, 2H, C**10**-H and C**5**-H), 2.14 (m, 1H, C**2**-H), 1.76 (m, 3H, C**4**-H and C**3**-CH_2), 1.36 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8 (C**11**), 71.5 (C**6**), 70.3 (C**8**), 62.4 (C**5**), 58.8 (C**9**), 51.8 (C**13**), 50.8 (C**2**), 35.8 (C**10**), 25.8 (C**4**), 20.3 (C**3**); HRMS: (CI^+) Calculated for $C_{10}H_{18}NO_4$: 216.1236. Found $[M+H]^+$: 216.1240.

4.3.39. Preparation of 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetic acid (2.21)

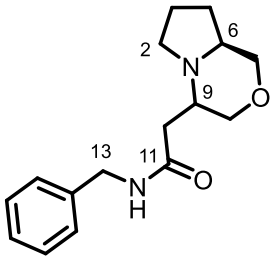


To a stirred solution of methyl ester **2.53.3** (0.90 g, 4.52 mmol, 1.0 eq.) in MeOH (30 mL) and H_2O (5 mL) was added LiOH (0.11 g, 4.52 mmol, 1.0 eq.) at rt, and the reaction was followed by TLC. Upon disappearance of starting material pH was adjusted to 5-6 using 2M HCl and solvents were removed *in vacuo*. The crude material

was purified by FCC (eluting with 3:2 EtOAc:MeOH) to afford the title compound (0.72 g, 86%) as a pale yellow foam.

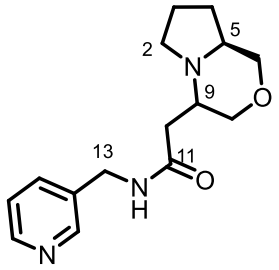
M.p. 130-133 ºC; *v* max/ cm^{-1} : 3321 (O-H) and 1756 (C=O); ^{1}H NMR (500 MHz, DMSO) δ 3.83 (dd, 1H, *J* = 5.0, 3.0 Hz, C**6**-H), 3.81 (dd, 1H, *J* = 5.5, 3.0 Hz, C**8**-H), 3.11 (td, 1H, *J* = 8.5, 4.0 Hz, C**2**-H), 3.07 (app. t, 1H, C**6**-H), 2.93 (app. t, 1H C**8**-H), 2.53 (m, 1H, C**9**-H), 2.29 (dd, 1H, *J* = 15.0, 4.5 Hz, C**10**-H), 2.03 (m, 1H, C**5**-H), 1.97 (m, 1H, C**2**-H), 1.81 (dd, 1H, *J* = 15.0, 8.6 Hz, C**10**-H), 1.67 (m, 1H, C**4**-H), 1.59 (m, 1H, C**3**-CH_2), 1.18 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, DMSO) δ 174.8 (C**11**), 71.0 (C**6**), 70.7 (C**8**), 62.6 (C**5**), 60.1 (C**9**), 50.6 (C**2**), 39.0 (C**10**), 26.0 (C**4**), 20.5 (C**3**); HRMS: (CI^+) Calculated for $C_9H_{15}NO_3Na$: 208.0950. Found $[M+Na]^+$: 208.0955.

4.3.40 Preparation of *N*-benzyl-2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetamide (2.55.1)



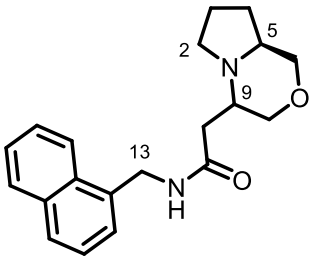
Following general procedure A; White crystals; yield: 102 mg, 69%; M.p. 145-149 ºC; *v* max/ cm^{-1} : 3317 (N-H), 1631 (C=O), 1168 (C-O) and 664 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (m, 5H, 5 x Ar-H), 4.37 (br. s, 2H, C**13**-CH_2), 3.94 (dd, 1H, *J* = 11.0, 3.0 Hz, C**8**-H), 3.79 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.20 (m, 3H, C**8**-H and C**2**-H and C**6**-H), 2.77 (m, 1H, C**9**-H), 2.52 (dd, 1H *J* = 14.5, 5.0 Hz, C**10**-H), 2.22 (m, 2H, C**10**-H and C**5**-H), 2.14 (m, 1H, C**2**-H), 1.81 (m, 1H, C**3**-H), 1.73 (m, 2H, C**4**-H and C**3**-H), 1.32 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 171.3 (C**11**), 138.4 (Ar), 128.1 (Ar), 127.4 (Ar), 126.9 (Ar), 70.5 (C**6**), 69.4 (C**8**), 62.7 (C**5**), 59.4 (C**9**), 50.3 (C**2**), 42.8 (C**13**), 36.7 (C**10**), 25.1 (C**4**), 19.9 (C**3**); HRMS: (CI^+) Calculated for $C_{16}H_{23}N_2O_2$: 275.1760. Found $[M+H]^+$: 275.1769.

4.3.41. Preparation of 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)-N-(pyridin-3-ylmethyl)acetamide (2.55.2)



Following general procedure A; Yellow oil; yield: 94 mg, 63%; *v* max/ cm^{-1} : 3287 (N-H), 1645 (C=O), 1189 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (m, 2H, 2 x Ar), 7.66 (dd, 1H *J* = 8.0, 1.5 Hz, Ar), 7.26 (dd, 1H, *J* = 8.0, 5.0 Hz, 1H, Ar), 4.43 (s, 2H, C**13**-CH_2), 3.99 (dd, 1H, *J* = 11.5, 3.0 Hz, C**8**-H), 3.85 (dd, 1H, *J* = 12.0, 3.5 Hz, C**6**-H), 3.48 (m, 1H, C**6**-H), 3.37 (m, 1H, C**8**-H), 3.16 (m, 1H, C**2**-H), 3.10 (m, 1H, C**9**-H), 2.62 (m, 2H, C**10**-CH_2), 2.55 (m, 1H, C**5**-H), 244 (m, 1H, C**2**-H), 1.90 (m, 1H, C**3**-H), 1.82 (m, 2H, C**3**-H and C**4**-H), 1.47 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7 (C**11**), 149.1 (Ar), 148.6 (Ar), 135.7 (Ar), 134.2 (Ar), 123.6 (Ar), 69.5 (C**6**), 68.2 (C**8**), 63.5 (C**5**), 59.2 (C**9**), 50.1 (C**2**), 40.9 (C**13**), 35.4 (C**10**), 25.0 (C**4**), 19.8 (C**3**); HRMS: (CI^+) Calculated for $C_{15}H_{22}N_3O_2$: 276.1712. Found $[M+H]^+$: 276.1720.

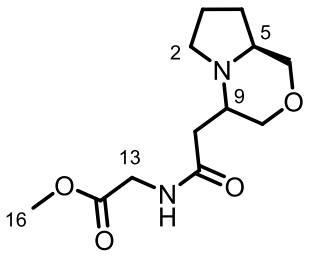
4.3.42. Preparation of 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)-N-(naphthalen-1-ylmethyl)acetamide (2.55.3)



Following general procedure A; Colourless viscous oil; yield: 175 mg, 59%; M.p. 160-163 ºC; *v* max/ cm^{-1} : 3378 (N-H), 1634 (C=O) and 1209 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (d, 1H, *J* = 8.0 Hz, Ar), 7.91 (d, 1H, *J* = 8.0 Hz, Ar), 7.84 (d, 1H, *J* = 8.0 Hz, Ar), 7.51 (m, 4H, 4 x Ar), 4.84 (s, 2H, C**13**-CH_2), 3.89 (dd, 1H, *J* = 11.0, 3.0 Hz, C**8**-H), 3.78 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.12 (m, 3H, C**8**-H and C**2**-H and

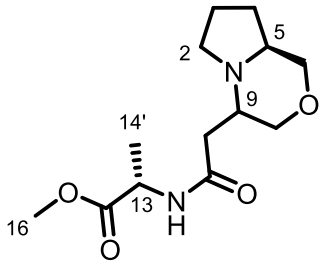
C**6**-H), 2.76 (m, 1H, C**9**-H), 2.47 (dd, 1H, *J* = 15.0, 5.0 Hz, C**10**-H), 2.20 (m, 2H, C**10**-H and C**5**-H), 2.09 (m, 1H, C**2**-H), 1.75 (m, 1H, C**3**-H), 1.62 (m, 2H, C**4**-H and C**3**-H), 1.19 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 171.1 (C**11**), 134.0 (Ar), 133.6 (Ar), 131.3 (Ar), 128.3 (Ar), 128.0 (Ar), 126.3 (Ar), 126.0 (Ar), 125.5 (Ar), 125.0 (Ar), 123.2 (Ar), 70.4 (C**6**), 69.2 (C**8**), 62.6 (C**5**), 59.3 (C**9**), 50.3 (C**2**), 40.9 (C**13**), 36.4 (C**10**), 24.9 (C**4**), 19.6 (C**3**); HRMS: (CI^+) Calculated for $C_{20}H_{25}N_2O_2$: 325.1916. Found $[M+H]^+$: 325.1918.

4.3.44. Preparation of methyl (2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)glycinate (2.56.1)



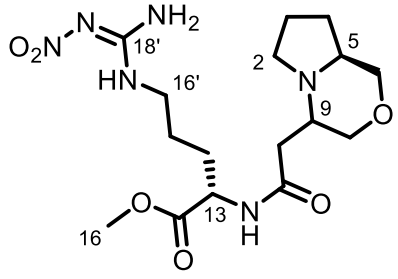
Following general procedure A; Pale yellow solid; yield: 0.16 g, 68%; M.p. 102-105 ºC; *v* max/ cm^{-1} : 3368 (N-H), 1703 (C=O) and 1632 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (s, 1H, NH), 4.12 (dd, 1H, *J* = 18.5, 5.5 Hz, C**13**-H), 4.02 (dd, 1H, *J* = 18.5, 5.5 Hz, C**13**-H), 3.98 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.78 (m, 1H, C**8**-H), 3.76 (s, 3H, C**16**-CH_3), 3.49 (app. t, 1H, *J* = 11.0 Hz, C**8**-H), 3.32 (m, 2H, C**6**-H and C**2**-H), 2.75 (ddt, 1H, *J* = 7.0, 5.5, 3.5 Hz, C**9**-H), 2.59 (dd, 1H, *J* = 17.0, 5.5 Hz, C**10**-H), 2.33 (dd, *J* = 17.0, 3.5 Hz, C**10**-H), 2.29 (m, 1H, C**5**-H), 2.19 (m, 1H, C**2**-H), 1.84 (m, 2H, C**1**-CH_2), 1.78 (m, 1H, C**4**-H), 1.43 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O), 170.4 (C=O), 70.9 (C**6**), 68.5 (C**8**), 62.5 (C**5**), 58.5 (C**9**), 52.2 (C**15**), 50.3 (C**2**), 41.0 (C**13**), 35.7 (C**10**), 25.7 (C**4**), 20.2 (C**3**); HRMS: (ES^+) Calculated for $C_{12}H_{21}N_2O_4$: 257.1496. Found $[M+H]^+$: 257.1501.

4.3.45. Preparation of methyl (2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-L-alaninate (2.56.2)



Following general procedure B; Pale yellow solid; yield: 0.06 g, 64%; M.p. 110-114 ºC; *v* max/ cm^{-1} : 1716 and 1635 (C=O) and 1203 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (s, 1H, NH), 4.61 (p, 1H, *J* = 7.0 Hz, C**13**-H), 4.00 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.80 (dd, 1H, *J* = 11.5, 3.5 Hz, C**8**-H), 3.75 (s, 3H, C**16**-CH_3), 3.41 (app. t, 1H, *J* = 11.0 Hz, C**8**-H), 3.36 (dd, 1H, *J* = 9.5, 8.5 Hz, C**2**-H), 3.28 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 2.77 (m, 1H, C**9**-H), 2.54 (dd, 1H, *J* = 16.5, 5.5 Hz, C**10**-H), 2.32 (m, 2H, C**10**-H and C**5**-H), 2.19 (q, 1H, *J* = 8.5 Hz, C**2**-H), 1.85 (m, 2H, C**3**-CH_2), 1.78 (m, 1H, C**4**-H), 1.44 (m, 4H, C**4**-H and C**14'**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4 (C=O), 169.7 (C=O), 71.1 (C**6**), 68.7 (C**8**), 62.4 (C**5**), 58.4 (C**9**), 52.3 (C**16**), 50.3 (C**2**), 47.8 (C**13**), 35.8 (C**10**), 25.7 (C**4**), 20.2 (C**3**), 18.5 (C**14'**); HRMS: (ES^+) Calculated for $C_{13}H_{23}N_2O_4$: 271.1658. Found $[M+H]^+$: 271.1665.

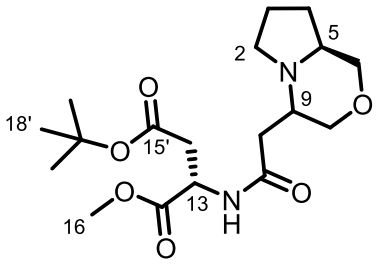
4.3.46. Preparation of methyl (*Z*)-N2-(2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-Nw'-nitro-*L*-argininate (2.56.3)



Following general procedure B; White foam; yield: 0.10 g, 48%; M.p. 152-156 ºC; *v* max/ cm^{-1} : 3297 (N-H), 1736 (C=O), 1624 (N=O) and 1251 (C-H); ^{1}H NMR (500 MHz, CD_3OD) δ 4.45 (dd, 1H, *J* = 8.5, 5.0 Hz, C**13**-H), 3.96 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.81 (dd, 1H, *J* = 11.5, 3.5 Hz, C**8**-H), 3.74 (s, 3H, C**16**-CH_3), 3.29 (m,

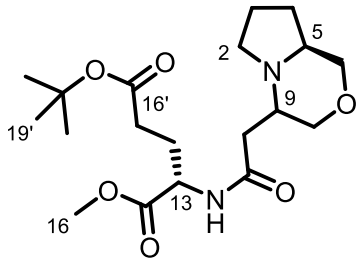
4H, C**6**-H, C**2**-H and C**16'**-CH_2), 3.21 (m, 1H, C**8**-H), 2.77 (m, 1H, C**9**-H), 2.53 (dd, 1H, *J* = 15.0, 5.0 Hz, C**10**-H), 2.25 (m, 2H, C**10**-H and C**5**-H), 2.16 (app. q, 1H, *J* = 9.0 Hz, C**2**-H), 1.93 (m, 1H, C**15'**-H), 1.81 (m, 5H, C**14'**-CH_2, C**4**-H and C**3**-CH_2), 1.70 (m, 1H, C**15'**-H), 1.36 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, CD_3OD) δ 172.2 (C=O), 171.7 (C=O), 129.8 (C**18'**), 70.5 (C**6**), 69.3 (C**8**), 62.6 (C**5**), 59.1 (C**9**), 51.9 (C**13**), 51.3 (C**16**), 50.3 (C**2**), 40.2 (C**16'**), 36.1 (C**10**), 28.2 (C**14'**), 25.0 (C**4** and C**15'**), 19.7 (C**3**); HRMS: (ES^+) Calculated for $C_{16}H_{29}N_6O_6$: 401.2149. Found $[M+H]^+$: 401.2139.

4.3.47. Preparation of 4-(*tert*-butyl) 1-methyl (2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-*L*-aspartate (2.56.4)



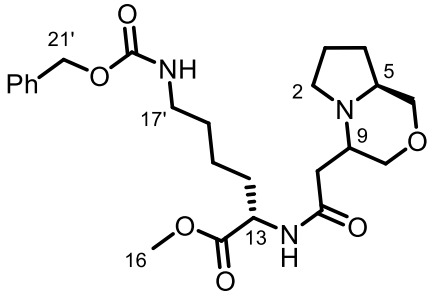
Following general procedure B; Yellow oil; yield: 0.18 g, 67%; *v* max/ cm^{-1} : 1706 and 1692 and 1652 (C=O); ^{1}H NMR (400 MHz, CD_3OD) δ 4.76 (m, 1H, C**13**-H), 3.95 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.78 (m, 1H, C**8**-H), 3.74 (s, 3H, C**16**-CH_3), 3.31 (m, 2H, C**2**-H and C**6**-H), 3.23 (dd, 1H, *J* = 15.5, 6.0 Hz, C**8**-H), 2.79 (m, 2H, C**14'**-CH_2), 2.74 (m, 1H, C**9**-H), 2.46 (dd, 1H, *J* = 15.5, 4.5 Hz, C**10**-H), 2.33 (dd, 1H, *J* = 15.5, 7.0 Hz, C**10**-H), 2.24 (m, 1H, C**5**-H), 2.17 (m, 1H, C**2**-H), 1.81 (m, 3H, C**4**-H and C**3**-CH_2), 1.47 (s, 9H, 2 x C**18'**-CH_3), 1.40 (m, 1H, C**4**-H); HRMS: (ES^+) Calculated for $C_{18}H_{31}N_2O_6$: 371.2182. Found $[M+H]^+$: 371.2180.

4.3.48. Preparation of 5-(*tert*-butyl) 1-methyl (2-((8aS)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-*L*-glutamate (2.56.5)



Following general procedure B; Yellow oil; yield: 0.23 g, 74%; *v* max/ cm^{-1} : 1712 and 1685 and 1629 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (s, 1H, NH), 4.63 (m, 1H, C**13**-H), 4.01 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.81 (dd, 1H, *J* = 11.0, 3.5 Hz, C**8**-H), 3.75 (s, 3H, C**16**-CH_3), 3.43 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 3.35 (dd, 1H, *J* = 9.5, 7.5 Hz, C**2**-H), 3.29 (app. t, 1H, *J* = 11.0 Hz, C**8**-H), 2.75 (m, 1H, C**9**-H), 2.57 (dd, 1H, *J* = 17.0, 6.0 Hz, C**10**-H), 2.32 (m, 4H, C**10**-H, C**5**-H and C**14'**-CH_2), 2.16 (m, 2H, C**15'**-CH_2), 1.99 (m, 1H, C**4**-H), 1.83 (m, 3H, C**3**-CH_2 and C**4**-H), 1.46 (s, 9H, 3 x C**19'**-CH_3), 1.42 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3 (C=O), 171.8 (C=O), 170.0 (C=O), 80.8 (C**18'**), 71.1 (C**6**), 68.8 (C**8**), 62.4 (C**5**), 58.4 (C**9**), 52.3 (C**16**), 51.4 (C**2**), 50.4 (C**13**), 35.8 (C**10**), 31.5 (C**14'**), 28.0 (C**19'**), 27.5 (C**15'**), 25.7 (C**4**), 20.2 (C**3**); HRMS: (ES^+) Calculated for $C_{19}H_{33}N_2O_6$: 385.2339. Found $[M+H]^+$: 385.2341.

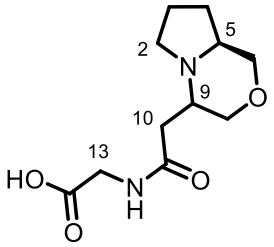
4.3.49. Preparation of methyl N6-((benzyloxy)carbonyl)-N2-(2-((8aS)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-*L*-lysinate (2.56.6)



Following general procedure B; Pale yellow viscous oil; yield: 0.22 g, 92%; *v* max/ cm^{-1} : 3387 (N-H), 1722 (C=O) and 1698 (C=O) and 1209 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H, NH), 7.35 (m, 5H, Ar), 5.11 (s, 2H, C**21'**-CH_2), 4.89 (s, 1H, NH), 4.62 (td, 1H, *J* = 7.5, 5.0 Hz, C**13**-H), 4.00 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H),

3.78 (dd, 1H, *J* = 11.5, 3.5 Hz, C**8**-H), 3.73 (s, 3H, C**16**-CH_3), 3.42 (app. t, 1H, *J* = 11.0 Hz, C**2**-H), 3.36 (m, 1H, C**6**-H), 3.27 (m, 1H, C**8**-H), 3.21 (m, 2H, C**17'**-CH_2), 2.76 (m, 1H, C**9**-H), 2.56 (dd, 1H, *J* = 17.0, 5.5 Hz, C**10**-H), 2.31 (m, 2H, C**10**-H and C**5**-H), 2.18 (app. q, 1H, *J* = 9.0 Hz, C**2**-H), 1.85 (m, 3H, C**4**-H and C**16'**-CH_2), 1.77 (m, 2H, C**3**-CH_2), 1.57 (m, 2H, C**15'**-CH_2), 1.41 (m, 3H, C**4**-H and C**14'**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7 (C=O), 170.0 (C=O), 156.4 (C**19'**), 136.5 (Ar), 128.5 (Ar), 128.0 (Ar), 71.1 (C**6**), 68.9 (C**8**), 66.6 (C**17'**), 62.4 (C**5**), 58.4 (C**9**), 52.2 (C**2**), 51.3 (C**16**), 50.3 (C**13**), 40.6 (C**21'**), 35.7 (C**10**), 32.0 (C**14'**), 29.4 (C**16'**), 25.7 (C**4**), 22.5 (C**15'**), 20.2 (C**3**); HRMS: (ES^+) Calculated for $C_{24}H_{36}N_3O_6$: 462.2599. Found $[M+H]^+$: 462.2600.

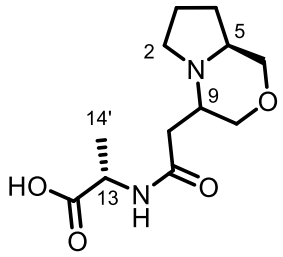
4.3.50. Preparation of (2-((8aS)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)glycine (2.57.1)



To a stirred solution of methyl ester **2.56.1** (0.05 g, 0.20 mmol, 1.0 eq.) in MeOH (2 mL) and H_2O (0.5 mL) was added LiOH (0.005 g, 0.20 mmol, 1.0 eq.) at rt, and the reaction was followed by TLC. Upon disappearance of starting material pH was adjusted to 5-6 using 2M HCl and solvents were removed *in vacuo*. The crude material was purified by FCC (eluting with 3:2 EtOAC:MeOH) to afford the title compound (0.033 g, 70%) as a yellow semi solid.

v max/ cm^{-1} : 3328 (N-H), 1759 (C=O) and 1206 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 4.02 (dd, 1H, *J* = 11.5, 3.0 Hz, C**6**-H), 3.95 (dd, 1H, *J* = 12.0, 3.0 Hz, C**8**-H), 3.79 (m, 2H, C**13**-H), 3.46 (m, 1H, C**2**-H), 3.41 (m, 1H, C**6**-H), 3.37 (m, 1H, C**8**-H), 3.14 (m, 1H, C**9**-H), 2.69 (m, 1H, C**5**-H), 2.62 (dd, 1H, *J* = 15.0, 5.5 Hz, C**10**-H), 2.54 (m, 1H, C**2**-H), 2.38 (dd, 1H, *J* = 15.0, 7.5 Hz, C**10**-H), 1.96 (m, 1H, C**4**-H), 1.90 (m, 2H, C**3**-CH_2), 1.50 (m, 1H, C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 170.4 (C=O), 69.1 (C**6**), 68.2 (C**8**), 63.2 (C**5**), 59.2 (C**9**), 49.6 (C**2**), 42.7 (C**13**), 35.4 (C**10**), 24.5 (C**4**), 19.4 (C**3**); HRMS: (CI^+) Calculated for $C_{11}H_{18}N_2O_4Na$: 265.1644. Found $[M+Na]^+$: 265.1645.

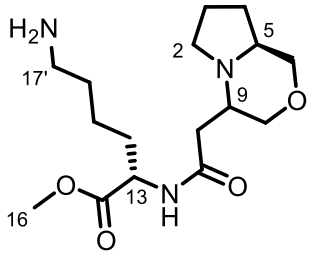
4.3.51. Preparation of (2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-*L*-alanine (2.57.2)



To a stirred solution of ester **2.56.2** (0.05 g, 0.19 mmol, 1.0 eq.) in MeOH (2 mL) and H_2O (0.5 mL) was added LiOH (0.005 g, 0.19 mmol, 1.0 eq.) at rt, and the reaction was followed by TLC. Upon disappearance of starting material pH was adjusted to 5-6 using 2M HCl and solvents were removed *in vacuo*. The crude material was purified by FCC (eluting with 3:2 EtOAc:MeOH) to afford the title compound (0.020 g, 42%) as a yellow semi solid.

v max/ cm^{-1} : 3386 (N-H), 1749 (C=O) and 1196 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 4.25 (q, 1H, *J* = 7.0 Hz, C**13**-H), 4.04 (dd, 1H, *J* = 11.5, 3.0 Hz, C**6**-H), 3.93 (dd, 1H, *J* = 11.5, 3.0 Hz, C**8**-H), 3.53 (m, 1H, C**2**-H), 3.46 (app. t, 1H *J* = 11.0 Hz, C**6**-H), 3.40 (app. t, *J* = 11.5 Hz, C**8**-H), 3.23 (m, 1H, C**9**-H), 2.80 (m, 1H, C**5**-H), 2.67 (m, 1H, C**10**-H), 2.62 (dd, 1H, *J* = 18.0, 9.0 Hz, C**2**-H), 2.38 (dd, 1H, *J* = 15.0, 7.5 Hz, C**10**-H), 2.00 (m, 1H, C**4**-H), 1.93 (m, 2H, C**3**-CH_2), 1.54 (m, 1H, C**4**-H), 1.38 (d, 3H, *J* = 7.0 Hz, C**14'**-CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 169.7 (C=O), 68.8 (C**6**), 67.9 (C**8**), 63.4 (C**5**), 59.1 (C**9**), 50.1 (C**2**), 49.6 (C**13**), 35.1 (C**10**), 24.4 (C**4**), 19.4 (C**3**), 17.1 (C**14'**); HRMS: (CI^+) Calculated for $C_{12}H_{20}N_2O_4Na$: 279.1321. Found $[M+Na]^+$: 279.1325.

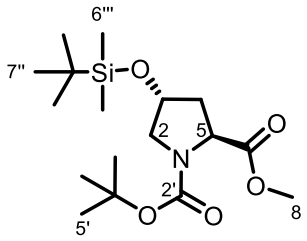
4.3.52. Preparation of methyl (2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetyl)-*L*-lysinate (2.58)



To a stirred solution of Cbz protected amine **2.56.6** (60 mg, 0.013 mmol, 1.0 eq.) in methanol (5 mL) was added 10 % Pd/C (cat.). The reaction was placed under a H_2 atmosphere and stirred overnight. The reaction solution was filtered through a pad of celite and solvents removed *in vacuo* to afford the title compound (43 mg, quant.) as a yellow semi solid.

v max/ cm^{-1} : 3369 and 3346 (N-H), 1687 and 1623 (C=O) and 1246 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 4.41 (dd, 1H, *J* = 9.0, 5.0 Hz, C**13**-H), 4.03 (dd, 1H, *J* = 11.5, 3.0 Hz, C**6**-H), 3.88 (dd, 1H, *J* = 12.0, 3.0 Hz, C**8**-H), 3.74 (s, 3H, C**16**-CH_3), 3.42 (m, 1H, C**2**-H), 3.33 (m, 2H, C**6**-H and C**8**-H), 3.04 (m, 1H, C**9**-H), 2.96 (t, 2H, *J* = 7.5 Hz, C**17'**-H), 2.65 (dd, 1H, *J* = 15.0, 5.5 Hz, C**10**-H), 2.58 (m, 1H, C**2**-H), 2.43 (m, 1H, C**5**-H), 2.35 (dd, 1H, *J* = 15.0, 7.0 Hz, C**10**-H), 1.91 (m, 4H, C**3**-CH_2 and C**15**'-CH_2), 1.78 (m, 1H, C**14**-H), 1.71 (m, 1H, C**14**-H), 1.48 (m, 3H, C**4**-H and C**16'**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 172.3 (C=O), 171.1 (C=O), 69.5 (C**6**), 68.6 (C**8**), 63.1 (C**5**), 59.1 (C**9**), 52.1 (C**2**), 51.3 (C**16**), 50.1 (C**13**), 39.0 (C**17'**), 35.4 (C**10**), 30.4 (C**14'**), 26.6 (C**16'**), 24.7 (C**4**), 22.5 (C**15'**), 19.5 (C**3**); HRMS: (ES^+) Calculated for $C_{16}H_{30}N_3O_4$: 328.2236. Found $[M+H]^+$: 328.2240.

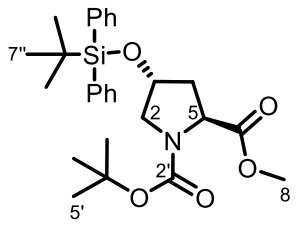
4.3.53. Preparation of 1-(*tert*-butyl) 2-methyl (2*S*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)pyrrolidine-1,2-dicarboxylate (2.60.1)



To a stirred solution of boc-4-hydroxy-*L*-proline methyl ester (1.22 g, 4.98 mmol, 1.0 eq.) and imidazole (0.75 g, 10.95 mmol, 2.2 eq.) in DMF (8 mL) was added TBDMS chloride (0.90 g, 5.98 mmol, 1.2 eq.). The reaction was allowed to stir at ambient temperature and progress was followed by TLC. Upon loss of starting material the reaction mixture was diluted with ether (50 mL) and washed with 1M HCl (50 mL) and saturated sodium bicarbonate solution (50 mL), dried ($MgSO_4$) and solvents were removed *in vacuo*. The crude material was purified by FCC (eluting with 1:9 EtOAc:hexanes) to afford the title compound (1.49 g, 83%) as a colourless oil.

v max/ cm^{-1} : 1748 and 1697 (C=O), 1104 and 1088 (C-O) and 699 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.43 (m, 2H, C**3**-H and C**3**-H), 3.75 (s, 3H, C**8**-CH_3), 3.61 (m, 1H, C**2**-H), 3.37 (m, 1H, C**2**-H), 2.19 (m, 1H, C**4**-H), 2.04 (m, 1H, C**4**-H), 1.45 (app. d, 9H, C**5'**-H), 0.91 (app. d, 9H, C**7''**-H), 0.10 (app. d, 6H, C**6'''**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8 (C**6**), 153.9 (C**2'**), 80.1 (C**4'**), 69.7 (C**3**), 58.1 (C**5**), 54.6 (C**2**), 52.0 (C**8**), 39.8 (C**4**), 28.3 (C**5'**), 25.7 (C**7''**), 18.0 (C**6''**), -4.9 (C**6'''**); HRMS: (CI^+) Calculated for $C_{17}H_{34}NO_5Si$: 360.2206. Found $[M+H]^+$: 360.2211.

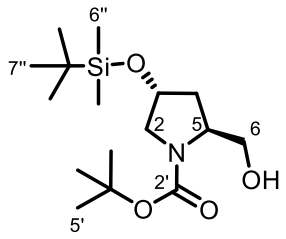
4.3.54. Preparation of 1-(*tert*-butyl) 2-methyl (2*S*,4*R*)-4-((*tert*-butyldiphenylsilyl)oxy)pyrrolidine-1,2-dicarboxylate (2.60.2)



To a stirred solution of boc-4-hydroxy-*L*-proline methyl ester (1.20 g, 4.90 mmol, 1.0 eq.) and imidazole (0.73 g, 10.77 mmol, 2.2 eq.) in DMF (8 mL) was added TBDPS chloride (1.61 g, 5.87 mmol, 1.2 eq.). The reaction was allowed to stir at ambient temperature and progress was followed by TLC. Upon loss of starting material the reaction mixture was diluted with ether (50 mL) and washed with 1M HCl (50 mL) and saturated sodium bicarbonate solution (50 mL), dried ($MgSO_4$) and solvents were removed *in vacuo*. The crude material was purified by FCC (eluting with 1:9 EtOAc:hexanes) to afford the title compound (2.35 g, 99%) as a viscous colourless oil.

v max/ cm^{-1} : 1749 and 1699 (C=O), 1364 (C-H) and 1154 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (m, 4H, 4 x C**7'''**-H), 7.41 (m, 6H, 2 x C**9'''**-H and 4 x C**8'''**-H), 4.40 (m, 2H, C**3**-H and C**5**-H), 3.67 (s, 3H, C**8**-H), 3.51 (m, 1H, C**2**-H), 3.41 (m, 1H, C**2**-H), 2.23 (m, 1H, C**4**-H), 1.86 (m, 1H, C**4**-H), 1.44 (d, 9H, 3 x C**5'**-CH_3), 1.05 (s, 9H, 3 x C**7''**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6 (C**6**), 154.7 (C**2'**), 135.6 (C**7'''**), 133.5 (C**6'''**), 129.9 (C**9'''**), 128.0 (C**8'''**), 80.1 (C**4'**), 70.8 (C**3**), 58.2 (C**5**), 54.5 (C**2**), 51.9 (C**8**), 38.8 (C**4**), 28.3 (C**5'**), 26.8 (C**7''**), 19.1 (C**6''**); HRMS: (CI^+) Calculated for $C_{27}H_{38}NO_5Si$: 484.2519. Found $[M+H]^+$: 484.2516.

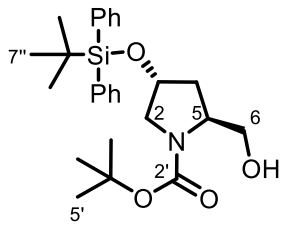
4.3.55. Preparation of tert-butyl (2*S*,4*R*)-4-((tert-butyldimethylsilyl)oxy)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (2.61.1)



To a stirred solution of ester **2.60.1** (1.45 g, 4.04 mmol, 1.0 eq.) in THF (8 mL) at 0 °C was added $LiBH_4$ (0.20 mL, 6.06 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL), cooled to 0 °C and 1M HCl (50 mL) was added. The organic phase was collected and washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$) and solvents removed *in vacuo* to afford the title compound (1.29 g, 96%) as a colourless oil.

v max/ cm^{-1} : 3421 (O-H), 1695 (C=O) and 1162 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.89 (app. d, 1H, OH), 4.29 (s, 1H, C**3**-H), 4.15 (m, 1H, C**5**-H), 3.71 (m, 1H, C**6**-H), 3.56 (m, 1H, C**6**-H), 3.45 (m, 1H, C**2**-H), 3.36 (m, 1H, C**2**-H), 1.98 (m, 1H, C**4**-H), 1.60 (m, 1H, C**4**-H), 1.47 (app. d, 9H, 3 x C**5'**-CH_3), 0.89 (s, 9H, 3 x C**7''**-CH_3), 0.08 (s, 6H, 2 x C**6'''**-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.5 (C**2'**), 80.4 (C**4'**), 69.8 (C**3**), 67.4 (C**6**), 59.1 (C**5**), 56.1 (C**2**), 38.0 (C**4**), 28.4 (C**5'**), 25.7 (C**7''**), 17.9 (C**6''**), -4.9 (C**6'''**); HRMS: (CI^+) Calculated for $C_{16}H_{34}NO_4Si$: 332.2257. Found $[M+H]^+$: 332.2262.

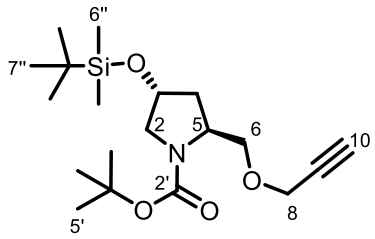
4.3.56. Preparation of tert-butyl (*2S*,*4R*)-4-((tert-butyldiphenylsilyl)oxy)-2-(hydroxymethyl)pyrrolidine-1-carboxylate (2.61.2)



To a stirred solution of ester **2.60.2** (2.30 g, 4.77 mmol, 1.0 eq.) in THF (10 mL) at 0 °C was added $LiBH_4$ (0.16 g, 7.14 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL), cooled to 0 °C and 1M HCl (50 mL) was added. The organic phase was collected and washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$) and solvents removed *in vacuo.* The crude material was purified by FCC (eluting with 1:3 EtOAc:hexanes) to afford the title compound (2.00 g, 92%) as a colourless oil.

v max/ cm^{-1} : 3486 (O-H), 1668 (C=O), 1105 (C-O) and 700 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (m, 4H, 4 x C**7'''**-H), 7.41 (m, 6H, 2 x C**9'''**-H and 4 x C**8'''**-H), 4.90 (app. d, 1H, OH), 4.24 (m, 2H, C**3**-H and C**5**-H), 3.65 (m, 1H, C**6**-H), 3.48 (m, 2H, C**2**-H and C**6**-H), 3.15 (m, 1H, C**2**-H), 2.02 (m, 1H, C**4**-H), 1.46 (br. s, 10H, C**4**-H and 3 x C**5'**-CH_3), 1.05 (s, 9H, 3 x C**7''**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.5 (C**2'**), 135.6 (C**7'''**), 133.6 (C**6'''**), 129.9 (C**9'''**), 127.8 (C**8'''**), 80.4 (C**4'**), 70.9 (C**3**), 67.4 (C**6**), 59.2 (C**5**), 55.9 (C**2**), 37.7 (C**4**), 28.4 (C**5'**), 26.8 (C**7''**), 19.1 (C**6''**); HRMS: (CI^+) Calculated for $C_{26}H_{38}NO_4Si$: 456.2570. Found $[M+H]^+$: 456.2571.

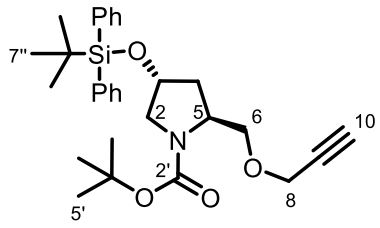
4.3.57. Preparation of *tert*-butyl (2S,4R)-4-((tert-butyldimethylsilyl)oxy)-2-((prop-2-yn-1-yloxy)methyl)pyrrolidine-1-carboxylate (2.63.1)



To a stirred solution of alcohol **2.61.1** (1.25 g, 3.77 mmol, 1.0 eq.) in THF (20 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.18 g, 4.53 mmol, 1.2 eq.). The reaction mixture was allowed to stir at 0 °C for 20 minutes followed by the dropwise addition of propargyl bromide (80% wt. in toluene, 0.51 mL, 4.53 mmol, 1.2 eq.) the reaction was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was diluted with EtOAC (50 mL) and washed with H2O (50 mL), saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and solvents removed *in vacuo.* The crude material was purified by FCC (eluting with 1:9 EtOAc:hexanes) to afford the title compound (1.07 g, 77%) as a yellow oil.

v max/ cm^{-1} : 2265 (C≡C), 1701 (C=O) and 1124 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40 (m, 1H, C**3**-H), 4.12 (m, 3H, C**5**-H and C**8**-CH_2), 3.58 (m, 2H, C**6**-CH_2), 3.34 (m, 2H, C**2**-CH_2), 2.41 (m, 1H, C**10**-H), 2.05 (dt, *J* = 12.5, 5.5 Hz, 1H, C**4**-H), 1.96 (m, 1H, C**4**-H), 1.46 (s, 9H, 3 x C**5'**-CH_3), 0.87 (s, 9H, 3 x C**7''**-CH_3), 0.06 (app. d, 6H, 2 x C**6'''**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8 (C**2'**), 79.8 (C**4'**), 79.4 (C**9**), 74.2 (C**10**), 71.4 (C**6**), 70.8 (C**3**), 58.5 (C**8**), 55.6 (C**5**), 55.1 (C**2**), 38.7 (C**4**), 28.5 (C**5'**), 25.7 (C**7'''**), 18.0 (C**6''**), -4.9 (C**6'''**); HRMS: (CI^+) Calculated for $C_{19}H_{35}NO_4Si$: 369.2335. Found $[M+H]^+$: 369.2332.

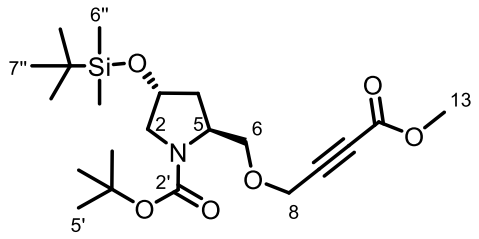
4.3.58. Preparation of *tert*-butyl (2*S*,4*R*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-((prop-2-yn-1-yloxy)methyl)pyrrolidine-1-carboxylate (2.63.2)



To a stirred solution of alcohol **2.61.2** (1.70 g, 3.73 mmol, 1.0 eq.) in THF (20 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.18 g, 4.48 mmol, 1.2 eq.). The reaction mixture was allowed to stir at 0 °C for 20 minutes followed by the dropwise addition of propargyl bromide (80% wt. in toluene, 0.50 mL, 4.48 mmol, 1.2 eq.) the reaction was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was diluted with EtOAC (50 mL) and washed with H2O (50 mL), saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and solvents removed *in vacuo.* The crude material was purified by FCC (eluting with 1:9 EtOAc:hexanes) to afford the title compound (1.12 g, 61%) as a colourless oil.

v max/ cm^{-1} : 2125 (C≡C), 1691 (C=O) and 1106 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 4H, 2 x C**7'''**-H), 7.41 (m, 6H, 2 x C**9'''**-H and 4 x C**8'''**-H), 4.38 (m, 1H, C**3**-H), 4.09 (m, 3H, C**5**-H and C**8**-CH_2), 3.55 (m, 2H, C**6**-CH_2), 3.40 (m, 1H, C**2**-H), 3.21 (m, 1H, C**2**-H), 2.33 (m, 1H, C**10**-H), 2.04 (m, 1H, C**4**-H), 1.93 (m, 1H, C**4**-H), 1.46 (s, 9H, 3 x C**5'**-CH_3), 1.04 (s, 9H, 3 x C**7''**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7 (C**2'**), 135.7 (Ar), 133.9 (C**6'''**), 129.8 (C**9'''**), 127.7 (Ar), 79.4 (C**4'**), 77.2 (C**9**), 74.2 (C**10**), 71.4 (C**3**), 70.7 (C**6**), 58.4 (C**8**), 55.6 (C**5**), 54.5 (C**2**), 38.4 (C**4**), 28.5 (C**5'**), 26.8 (C**7''**), 19.1 (C**6''**); HRMS: (CI^+) Calculated for $C_{29}H_{40}NO_4Si$: 494.2727. Found $[M+H]^+$: 494.2735.

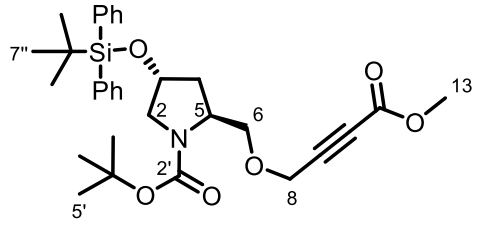
4.3.59. Preparation of tert-butyl (2*S*,4*R*)-4-((tert-butyldimethylsilyl)oxy)-2-(((4-methoxy-4-oxobut-2-yn-1-yl)oxy)methyl)pyrrolidine-1-carboxylate (2.64.1)



To a stirred solution of propargyl ether **2.63.1** (1.05 g, 2.84 mmol, 1.0 eq.) in THF (8 mL) at -78 °C was added *n*-BuLi (2.5M in hexanes, 1.37 mL, 3.41 mmol, 1.2 eq.) dropwise. The reaction mixture was allowed to stir at -78 °C for 30 minutes followed by the dropwise addition of methyl chloroformate (0.32 mL, 3.41 mmol, 1.2 eq.). The reaction mixture was allowed to warm to ambient temperature and followed by TLC. Upon completion the reaction mixture was diluted with EtOAc (50 mL) and washed with H2O (50 mL), saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.81 g, 67%) as a yellow oil.

v max/ cm^{-1} : 1719 and 1656 (C=O) and 1162 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.38 (p, *J* = 4.8 Hz, 1H), 4.34 – 4.21 (m, 2H), 4.16 – 3.95 (m, 1H), 3.78 (s, 3H), 3.64 (d, *J* = 6.5 Hz, 2H), 3.59 – 3.23 (m, 3H), 2.03 (dt, *J* = 12.5, 5.5 Hz, 1H), 1.96 (s, 1H), 1.46 (s, 9H), 0.87 (s, 9H), 0.06 (d, *J* = 1.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7 (C**2'**), 153.5 (C**11**), 83.5 (C**9**), 79.5 (C**10**), 71.5 (C**4**), 70.3 (C**6**), 70.0 (C**3**), 58.3 (C**8**), 55.6 (C**5**), 55.2 (C**2**), 52.8 (C**13**), 37.6 (C**4**), 28.5 (C**5'**), 25.7 (C**7''**), 18.0 (C**4'**), -4.9 (C**6'''**); HRMS: (CI^+) Calculated for $C_{21}H_{38}NO_6Si$: 428.2468. Found $[M+H]^+$: 428.2470.

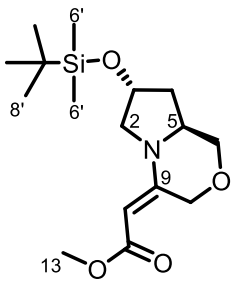
4.3.60. Preparation of *tert*-butyl (2*S*,4*R*)-4-((*tert*-butyldiphenylsilyl)oxy)-2-(((4-methoxy-4-oxobut-2-yn-1-yl)oxy)methyl)pyrrolidine-1-carboxylate (2.64.2)



To a stirred solution of propargyl ether **2.63.2** (1.05 g, 2.84 mmol, 1.0 eq.) in THF (8 mL) at -78 °C was added *n*-BuLi (2.5M in hexanes, 1.02 mL, 2.55mmol, 1.2 eq.) dropwise. The reaction mixture was allowed to stir at -78 °C for 30 minutes followed by the dropwise addition of methyl chloroformate (0.20 mL, 2.55 mmol, 1.2 eq.). The reaction mixture was allowed to warm to ambient temperature and followed by TLC. Upon completion the reaction mixture was diluted with EtOAc (50 mL) and washed with H2O (50 mL), saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.81 g, 68%) as a yellow oil.

v max/ cm^{-1} : 2189 (C≡C), 1698 and 1633 (C=O), 1161 (C-O) and 701 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (m, 4H, 2 x C**7'''**-H), 7.41 (m, 6H, 2 x C**9'''**-H and 4 x C**8'''**-H), 4.37 (m, 1H, C**3**-H), 4.15 (m, 3H, C**5**-H and C**8**-CH_2), 3.76 (s, 1H, C**13**-CH_3), 3.57 (m, 2H, C**6**-CH_2), 3.42 (m, 1H, C**2**-H), 3.20 (s, 1H, C**2**-H), 2.04 (s, 1H, C**4**-H), 1.91 (s, 1H, C**4**-H), 1.46 (s, 9H, 3 x C**5'**-CH_3), 1.04 (s, 9H, 3 x C**7''**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.1 (C**2'**), 153.5 (C**11**), 135.7 (C**7'''**), 133.8 (C**6'''**), 129.8 (C**9'''**), 127.7 (C**8'''**), 83.8 (C**4**), 79.5 (C**10**), 71.8 (C**4''**), 71.3 (C**3**), 70.7 (C**6**), 58.2 (C**5**), 55.6 (C**8**), 55.1 (C**2**), 52.7 (C**13**), 38.4 (C**4**), 28.5 (C**5'**), 26.8 (C**7''**), 19.1 (C**6''**); HRMS: (CI^+) Calculated for $C_{31}H_{42}NO_6Si$: 552.2781. Found $[M+H]^+$: 552.2785.

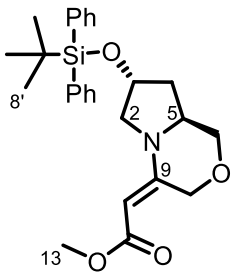
4.3.61. Preparation of methyl (*E*)-2-((7*R*,8a*S*)-7-((*tert*-butyldimethylsilyl)oxy) tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetate (2.66.1)



To a stirred solution of boc protected amine **2.64.1** (0.80 g, 1.87 mmol, 1.0 eq.) in DCM (10 mL) at 0 °C was added TFA (3 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 5 hours the solvents were removed *in vacuo*. The residue was taken up in H_2O (30 mL) and pH was adjusted to 9-10 with 2M NaOH and stirred for 20 minute. The aqueous was extracted with DCM (3 x 30 mL), and the combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:17 EtOAc:hexanes) to afford the title compound (0.16 g, 26%) as a yellow oil.

v max/ cm^{-1} : 1631 (C=O) and 1253 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.66 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.52 (t, 1H, *J* = 4.5 Hz, C**3**-H), 4.38 (s, 1H, C**10**-H), 4.20 (dd, 1H, *J* = 11.0, 4.0 Hz, C**6**-H), 3.94 (m, 1H, C**5**-H), 3.62 (s, 3H, C**13**-CH_3), 3.47 (dd, 1H, *J* = 12.0, 4.5 Hz, C**2**-H), 3.24 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 3.17 (app. d, 1H, *J* = 12.0 Hz, C**2**-H), 1.93 (dd, 1H, *J* = 12.0, 4.5 Hz, C**4**-H), 1.43 (m, 1H, C**4**-H), 0.91 (s, 9H, 3 x C**7'**-CH_3), 0.11 (s, 6H, 2 x C**6''**-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5 (C**11**), 157.2 (C**9**), 79.5 (C**10**), 69.0 (C**3**), 68.9 (C**8**), 65.8 (C**6**), 57.9 (C**2**), 53.9 (C**5**), 50.1 (C**13**), 38.5 (C**4**), 25.7 (C**7'**), 18.0 (C**6'**), -4.8 (C**6''**); HRMS: (CI^+) Calculated for $C_{16}H_{30}NO_4Si$: 328.1944. Found $[M+H]^+$: 328.1948.

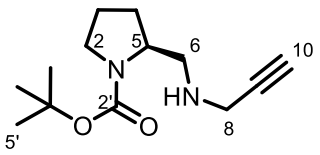
4.3.62. Preparation of methyl (*E*)-2-((7*R*,8a*S*)-7-((*tert*-butyldiphenylsilyl)oxy) tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetate (2.66.2)



To a stirred solution of boc protected amine **2.64.2** (0.80 g, 1.87 mmol, 1.0 eq.) in DCM (10 mL) at 0 °C was added TFA (3 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 5 hours the solvents were removed *in vacuo*. The residue was taken up in H_2O (30 mL) and pH adjusted to 9-10 with 2M NaOH and stirred for 20 minute. The aqueous was extracted with DCM (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo*. The residue was taken up in MeOH (10 mL) and stirred at reflux overnight before solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:17 EtOAc:hexanes) to afford the title compound (0.30 g, 49%) as a yellow oil.

v max/ cm^{-1} : 1637 C=O), 1471 (C=C) and 1151 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (m, 4H, Ar), 7.47 (m, 2H, C**9'**-H), 7.44j (m, 4H, Ar), 5.29 (d, 1H, *J* = 17.5 Hz, C**8**-H), 4.65 (d, 1H, *J* = 17.5, C**8**-H), 4.53 (t, 1H, *J* = 4.5 Hz, C**3**-H), 4.32 (s, 1H, C**10**-H), 4.20 (dd, 1H, *J* = 11.0, 4.0 Hz, C**6**-H), 4.07 (m, 1H, C**5**-H), 3.62 (s, 3H, C**13**-CH_3**)** 3.32 (dd, 1H, *J* = 12.5, 4.5 Hz, C**2**-H), 3.25 (d, 1H, *J* = 12.5 Hz, C**2**-H), 3.21 (app. t, 1H, *J* = 11.0 Hz, C**6**-H), 1.94 (dd, 1H, *J* = 12.5, 4.5 Hz, C**4**-H), 1.30 (m, 1H, C**4**-H), 1.09 (m, 9H, 3 x C**7''**-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5 (C**11**), 157.2 (C**9**), 135.7 (Ar), 133.3 (C**6'**), 130.0 (C**9'**), 127.9 (Ar), 79.5 (C**10**), 70.1 (C**3**), 68.9 (C**6**), 65.8 (C**8**), 57.6 (C**2**), 54.1 (C**5**), 50.1 (C**13**), 38.2 (C**4**), 26.9 (C**7''**), 19.1 (C**6''**); HRMS: (CI^+) Calculated for $C_{26}H_{34}NO_4Si$: 452.2257. Found $[M+H]^+$: 452.2264.

4.3.63. Preparation of tert-butyl *(S)*-2-((prop-2-yn-1-ylamino) methyl)pyrrolidine-1-carboxylate (2.69)

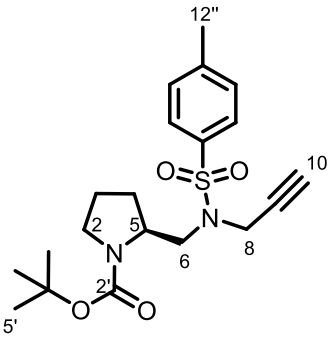


To a stirred solution of *N*-boc-*L*-prolinol (3.00 g, 14.9 mmol, 1.0 eq.) and DCM (50 mL) was added trichloroisocyanuric acid (3.81 g, 16.4 mmol, 1.1 eq.) and TEMPO (0.02 g, 0.15 mmol, 0.01 eq.) at 0 °C. The reaction mixture was left to stir at room temperature for 20 minutes. The reaction mixture was then filtered through celite, washed with sodium bicarbonate (20 mL), 1M HCl (20 mL) and brine (20 mL). The combined organic fractions were dried ($MgSO_4$) and solvents removed *in vacuo* to give *N*-benzylprolinal intermediate.

To a stirred solution of *N*-benzylprolinal (2.97 g, 14.9 mmol, 1.0 eq.) in DCM (75 mL) was added propargyl amine (1.44 mL, 22.5 mmol, 1.5 eq.), acetic acid (3.44 mL, 60 mmol, 4.0 eq.) and sodium triacetoxyborohydride (5.43 g, 25.65 mmol, 1.7 eq.) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight. The crude reaction mixture was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and reduced *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 2:3 EtOAc:Hexane) to afford the title compound (2.27 g, 64%) as a pale yellow oil.

v max /cm^{-1}: 3434 (N-H), 1655 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 – 3.95 (broad m, 1H, C**6**-H), 3.35 (d, 4H, C**8**-CH_2, C**2**-H and C**6**-H), 2.88 (broad m, 1H, C**5**-H), 2.68 (m, 1H, C**6**-H), 2.22 (broad m, 1H, C**10**-H), 1.92 (broad m, 5H, C**3**-CH_2, C**4**-CH_2 and C**2**-H), 1.47 (broad m, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7 (C=O), 82.3 (C**4'**), 80.1 (C**9**), 71.3 (C**10**), 57.1 (C**6**), 52.0 (C**5**), 46.6 (C**8**), 46.3 (C**2**), 38.3 (C**4**), 28.8 (C**5'**), 22.9 (C**3**); HRMS (ES+) m/z calculated for $C_{13}H_{23}N_2O_2$: 239.1697. Mass found $[M+H]^+$: 239.1701.

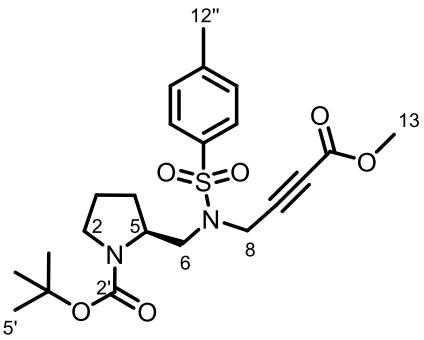
4.3.64. Preparation of *tert*-butyl *(S)*-2-(((4-methyl-*N*-(prop-2-yn-1-yl)phenyl)sulfonamido)methyl)pyrrolidine-1-carboxylate (2.70)



To a stirred solution of amine **2.69** (0.50 g, 2.51 mmol, 1.0 eq.) and triethylamine (0.38 mL, 3.77 mmol, 1.5 eq.) in DCM (30 mL) at 0 °C was added *p*-toluenesulfonyl chloride (0.57 g, 3.01 mmol, 1.2 eq.) dropwise. The reaction mixture was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was then diluted with H_2O (50 mL) and the solution extracted with DCM (3 x 30 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and reduced *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:9 EtOAc:hexane) to afford the title compound (0.54 g, 55%) as a white solid.

v max /cm^{-1}: 1689 (C=O), 1347 (S=O), 1160 (S=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (m, 2H, Ar), 7.28 (broad m, 2H, Ar), 4.15 (broad m, 2H, C**8**-CH_2), 4.02 (broad m, 2H, C**2**-CH_2), 3.35 (broad m, 1H, C**5**-H), 3.27 (m, 2H, C**6**-CH_2), 3.17 (broad m, 1H, C**10**-H), 2.42 (s, 3H, C**12''**-CH_3), 1.98 (broad m, 2H, C**4**-CH_2), 1.86 (broad m, 2H, C**3**-CH_2), 1.45 (broad s, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.5 (C**2'**), 135.9 (Ar), 129.4 (Ar), 128.1 (Ar), 127.8 (Ar), 79.9 (C**4'**), 79.3 (C**9**), 73.1 (C**10**), 55.5 (C**5**), 49.3 (C**2**), 48.4 (C**6**), 37.6 (C**8**), 28.4 (C**5'**), 27.8 (C**4**), 22.4 (C**3**), 21.5 (C**12'**); HRMS (ES+) calculated for $C_{20}H_{28}N_2O_4SNa$: 392.1803. Found $[M+Na]^+$: 415.1795;

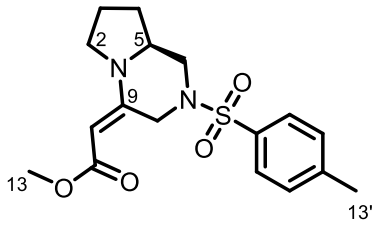
4.3.65. Preparation of tert-butyl *(S)*-2-(((*N*-(4-methoxy-4-oxobut-2-yn-1-yl)-4-methylphenyl)sulfonamido)methyl)pyrrolidine-1-carboxylate (2.71)



To a stirred solution of alkyne **2.70** (0.50 g, 1.27 mmol, 1.0 eq.) in THF (20 mL) was added *n*-BuLi (2.0 M in hexanes, 0.80 mL, 1.40 mmol, 1.1 eq.) at -78 °C. The reaction was allowed to stir at this temperature for a further 40 mins followed by the dropwise addition of methyl chloroformate (0.11 mL, 1.40 mmol, 1.1 eq.). The reaction was allowed to warm to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL) and H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (100 mL) and dried dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:7 EtOAc:hexane) to afford the title compound (0.18 g, 32%) as a white solid.

v max/ cm^{-1} : 1687 (C=O), 1348 (S=O) and 1160 (S=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (m, 2H, 2 x C**9''**-H), 7.32 (m, 2H, 2 x C**10''**-H), 4.30 (broad m, 2H, C**8**-H), 3.69 (s, 3H, C**13**-CH_3), 3.31 (broad m, 4H, C**2**-H_2, C**6**-CH_2), 2.04 (br. m, 1H, C**5**-H), 1.93 (broad m, 3H, C**12''**-CH_3), 1.56 (broad m, 4H, C**3**-CH_2, C**4**-CH_2), 1.44 (broad s, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5 (C**2'**), 155.4 (C**12''**), 141.2 (C**11''**), 139.3 (C**8''**), 129.8 (C**10''**), 129.6 (C**9''**), 79.4 (C**4'**), 68.4 (C**10**), 66.5 (C**9**), 65.8 (C**5**), 56.3 (C**13**), 49.7 (C**2**), 37.7 (C**6**), 34.1 (C**8**), 30.6 (C**4**), 28.0 (C**5'**), 24.3 (C**3**), 21.6 (C**11'**); HRMS: (ES+) Calculated for $C_{22}H_{30}N_2O_6SNa$: 473.1722. Found $[M+Na]^+$: 473.1726.

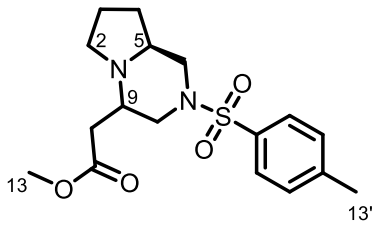
4.3.66. Preparation of methyl (*S*,*E*)-2-(2-tosylhexahydropyrrolo[1,2-a]pyrazin-4(1H)-ylidene)acetate (2.73)



To a stirred solution of Boc amine **2.71** (0.12 g, 0.26 mmol, 1.0 eq.) in DCM (10 mL) at 0 °C was added TFA (1.00 mL, XS) dropwise. The reaction was allowed to warm to ambient temperature and stirred for a further 3 hours and then solvents were remove *in vacuo*. The residue was taken up in H_2O (10 mL) and pH adjusted to 9-10 by addition of 2M NaOH. The aqueous solution was extracted with DCM (3 x 20 mL), the combined extracts were dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:7 EtOAc:hexane) to afford the title compound (0.05 g, 54%) as a white solid.

v max/ cm^{-1} : 1676 (C=O), 1345 (S=O) and 1145 (S=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 2H, 2 x Ar), 7.25 (m, 2H, 2 x Ar), 4.98 (s, 1H, C**10**-H), 4.05 (d, 1H, C**8**-H), 3.85 (d, 1H, C**8**-H), 3.15 (s, 3H, C**13**-CH_3), 3.15 (m, 1H, C**6**-H), 3.05 (m, 1H, C**6**-H), 2.35 (s, 3H, C**13'**-CH_3), 1.97 (m, 3H, C**2**-CH_2 and C**5**-H), 1.17 (m, 4H, C**3**-CH_2 and C**4**-CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 13C NMR (101 MHz, CDCl3) δ 168.4 (Ar), 153.7 (C**9**), 132.7 (Ar), 130.0 (Ar), 127.8 (C**10'**), 81.7 (C**10**), 57.6 (C**5**), 50.2 (C**6**), 48.7 (C**2**), 47.7 (C**13**), 45.2 (C**8**), 29.2 (C**4**), 22.3 (C**3**), 21.5 (C**13'**); HRMS: (ES+) Calculated for $C_{17}H_{23}N_2O_4S$: 351.1426 Found $[M+H]^+$: 351.1436.

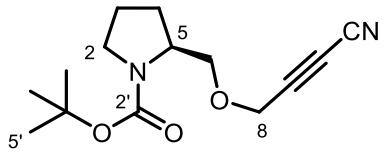
4.3.67. Preparation of methyl 2-((8a*S*)-2-tosyloctahydropyrrolo[1,2-a]pyrazin-4-yl)acetate (2.74)



To a stirred solution of *β*-enamino ester **2.73** (0.05 g, 0.14 mmol, 1.0 eq.) in MeOH (10 mL) was added 10 Pd/C (0.15 g, 0.01 mmol, 0.1 eq.). The reaction was placed under a H_2 atmosphere and stirred overnight. The reaction solution was filtered through a pad of celite and solvents removed *in vacuo* to afford the crude product. The crude material was purified by FCC (eluting with 1:1 EtOAc:hexane) to afford the title compound (0.04 g, 83%) as a white solid.

v max/ cm^{-1} : 1736 (C=O), 1345 (S=O) and 1163 (S=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 2H, 2 x Ar), 7.33 (d, 2H, 2 x Ar), 3.80 (m, 2H, C**6**-H and C**8**-H), 3.70 (s, 3H, C**13**-CH_3), 3.06 (m, 1H, C**8**-H), 2.77 (m, 1H, C**6**-H), 2.57 (m, 1H, C**9**-H), 2.43 (s, 3H, C**13'**-CH_3), 2.26 (m, 2H, C**10**-CH_2), 2.07 (m, 3H, C**2**-CH_2 and C**5**-H), 1.83 (m, 1H, C**3**-H), 1.72 (m, 2H, C**4**-CH_2), 1.59 (m, 1H, C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2 (Ar), 143.6 (C**9'**), 141.4 (Ar), 129.7 (Ar), 127.7 (Ar), 61.9 (C**5**), 58.4 (C**2**), 57.4 (C**9**), 51.9 (C**6**), 50.3 (C**8**), 49.7 (C**13**), 37.3 (C**10**), 27.0 (C**4**), 21.5 (C**3**), 20.6 (C**13'**); HRMS: (ES+): Calculated for $C_{17}H_{25}N_2O_4S$: 353.1535. Found $[M+H]^+$: 353.1536.

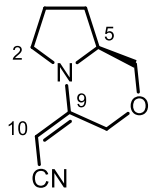
4.3.68. Preparation of *tert*-butyl *(S)*-2-(((3-cyanoprop-2-yn-1-yl)oxy)methyl) pyrrolidine-1-carboxylate (2.78)



To a stirred solution of alkyne **2.51** (1.00 g, 4.17 mmol, 1.0 eq.) in MeCN (20 mL) was added AIBN (1.03 g, 6.26 mmol, 1.5 eq.) and $CuI.3H_2O$ (0.20 g, 0.83 mmol, 0.20 eq.). The reaction was heated to reflux and stirred for 4 days. The solvents were then removed *in vacuo* and the residue taken up in H_2O (30 mL). The product was extracted with EtOAc (3 x 30 mL) and the combined organic extracts were dried ($MgSO_4$) and the crude material was purified by FCC (eluting with 1:9 EtOAc:hexane) to afford the title compound (0.31 g, 21%) as a pale yellow oil.

v max/ cm^{-1} : 2976 (C-H), 2302 (C≡N), 1686, (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (br. s, 2H, C**8**-CH_2), 3.92 (m, 1H, C**6**-H), 3.65 (br. m, 1H, C**6**-H), 3.55 (br. m, 1H, C**5**-H), 3.33 (br. m, 2H, C**2**-CH_2), 1.85 (br. m, 4H, C**4**-CH_2 and C**3**-CH_2), 1.47 (s, 9H, 3 x C**5'**-CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.7 (C**2'**), 125.4 (CN), 81.3 (C**4'**), 77.2 (C**10**), 71.8 (C**9**), 71.4 (C**8**), 58.1 (C**6**), 56.0 (C**5**), 46.5 (C**2'**), 28.7 (C**2**), 28.0 (C**4**), 23.8 (C**3'**); HRMS: (ES^+) Calculated for $C_9H_{13}N_2O$: 165.1028. Found $[M+H]^+$: 165.1029.

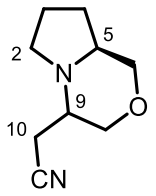
4.3.69. Preparation of (*S*,*E*)-2-(tetrahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4(3H)-ylidene)acetonitrile (2.79)



To a stirred solution of Boc protected amine **2.78** (0.29 g, 1.10 mmol, 1.0 eq.) in DCM (10 mL) at 0 °C was added TFA (3.00 mL). The reaction mixture was allowed to warm to room temperature and stirred for 3 hours. Solvents were then removed *in vacuo* and the residue was taken up in H_2O (30 mL). The pH was adjusted to 9-10 using 2M NaOH and stirred for a further 20 minute. The aqueous solution was then extracted with DCM (3 x 30 mL) and the combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:1 EtOAc:hexane) to afford the title compound (0.13 g, 74%) as a pale yellow oil.

v max/ cm^{-1} : 2185 (C≡N), 1586 (C=C), 1164 (C-O) and 1102 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.71 (d, 1H, *J* = 16.0 Hz, C**8**-H), 4.40 (1H, dd, 1H, *J* = 16.0, 1.5 Hz, C**8**-H), 4.20 (1H, dd, *J* = 11.0, 4.0 Hz, C**6**-H), 3.57 (s, 1H, C**10**-H), 3.50 (ddd, 1H, *J* = 15.0, 10.5, 4.5 Hz, C**5**-H), 3.26 (m, 2H, C**2**-H and C**6**-H), 3.17 (m, 1H, C**2**-H), 2.13 (m, 1H, C**3**-H), 2.02 (m, 1H, C**4**-H), 1.89 (m, 1H, C**3**-H), 1.36 (m, 1H, C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.3 (C**9**), 120. (CN), 69.7 (C**6**), 64.0 (C**8**), 56.1 (C**5**), 55.1 (C**10**), 47.2 (C**2**), 28.5 (C**4**), 22.3 (C**3**); HRMS: (ES^+) Calculated for $C_{14}H_{20}N_2NaO_3$: 287.1372. Found $[M+Na]^+$: 287.1368.

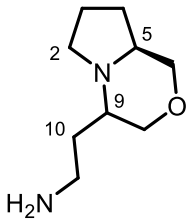
4.3.70. Preparation of 2-((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)acetonitrile (2.80)



To a stirred solution of *β*-enamino nitrile **2.79** (0.11 g, 0.67 mmol, 1.0 eq.) in EtOH (3 mL) was added sodium cyanoborohydride (0.05 g, 0.74 mmol, 1.1 eq.) and HCl (cat.). The reaction mixture was allowed to stir for a further 90 minutes at which time the reaction was diluted with H_2O (20 mL) and solution basified with ammonia solution. The aqueous solution as extracted with ether (5 x 20 mL) and the combined organic extracts were dried ($MgSO_4$) and solvents removed *in vacuo*. The crude material was purified by FCC (eluting with 1:1 EtOAc:hexane) to afford the title compound (0.085 g, 77%) as a pale yellow solid.

M.p. 113-116 ºC; *v* max/ cm^{-1} : 2246 (C≡N), 1162 (C-O) and 1094 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, 1H, *J* = 10.5, 3.0 Hz, C**6**-H), 3.90 (dd, 1H, *J* = 11.0, 3.0Hz, C**8**-H), 3.25 (m, 3H, C**2**-H and C**8**-H and C**6**-H), 2.60 (m, 1H, C**9**-H), 2.51 (dd, 1H, *J* = 17.0, 4.5 Hz, C**10**-H), 2.40 (1H, dd, 1H, *J* = 17.0, 7.0 Hz, C**10**-H), 2.24 (m, 1H, C**5**-H), 2.15 (m, 1H, C**2**-H), 1.78 (m, 3H, C**2**-H and C**3**-CH_2), 1.35 (m, 1H, C**2**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 117.0 (CN), 71.3 (C**6**), 69.5 (C**8**), 62.1 (C**5**), 57.9 (C**9**), 50.7 (C**2**), 25.6 (C**4**), 20.4 (C**3**), 19.2 (C**10**); HRMS: (ES^+) Calculated for $C_9H_{15}N_2O$: 167.1184. Found $[M+H]^+$: 167.1172.

4.3.71. Preparation of ((8a*S*)-hexahydro-1H-pyrrolo[2,1-c][1,4]oxazin-4-yl)methanamine (2.81)

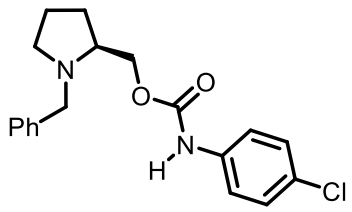


A solution of nitrile **2.80** (84 mg, 0.51 mmol, 1.0 eq.) in THF (3 mL) was added dropwise to a solution of $LiAlH_4$ (1 M in THF, 0.51 mL, 0.51 mmol, 1.0 eq.) in THF (10 mL) at 0 °C under a nitrogen atmosphere. The reaction was heated to reflux and stirred for a further 90 minutes, after which time the reaction mixture was cooled to ambient temperature and KOH (25 mg, 0.51 mmol, 1.0 eq.) in H_2O (3 mL) was added dropwise. The solution was filtered and solvents removed *in vacuo* to afford the title compound (54 mg, 63%) as an orange oil.

v max/ cm^{-1} : 3286 (N-H), 1121 (C-O) and 1069 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95 (dd, 1H, *J* = 11.0, 3.0 Hz, C**6**-H), 3.80 (dd, 1H, *J* = 11.0, 3.0 Hz, C**8**-H), 3.21 (m, 3H, C**2**-H and C**8**-H and C**6**-H), 2.73 (m, 2H, C**11**-CH_2), 2.33 (m, 1H, C**9**-H), 2.14 (m, 1H, C**5**-H), 2.05 (m, 1H, C**2**-H), 1.75 (m, 4H, C**4**-H and C**3**-CH_2 and C**10**-H), 1.45 (m, 1H, C**10**-H), 1.34 (m, 1H, C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.3 (C**6**), 70.3 (C**8**), 62.6 (C**5**), 60.6 (C**9**), 50.8 (C**2**), 38.9 (C**11**), 30.0 (C**10**), 25.8 (C**4**), 20.3 (C**3**); HRMS: (ES^+) Calculated for $C_9H_{18}N_2O$: 171.1497. Found $[M+H]^+$: 171.1484.

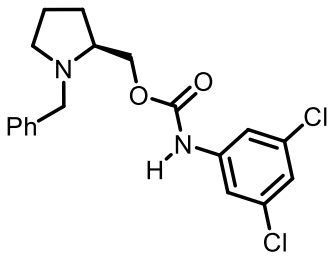
4.4. Individual Experimental Details for - Chapter 3

4.4.1. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl (4-chlorophenyl)carbamate (3.27.1)



Following general procedure B; Yellow oil; yield: 77%; *v* max/ cm^{-1} : 1655 (C=O), 1122 (C-O) and 698 (C-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32 (m, 5H, 5 x Ar), 7.25 (m, 4H, 4 x Ar), 6.62 (s, 1H, NH), 4.19 (dd, *J* = 11.0, 5.0 Hz, 1H, C**6**-H), 4.12 (dd, *J* = 11.0, 3.0 Hz, 1H, C**6**-H), 4.05 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 3.45 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 2.98 (m, 1H, C**2**-H), 2.87 (m, 1H, C**5**-H), 2.31 (m, 1H, C**2**-H), 1.99 (m, 1H, C**4**-H), 1.77 (m, 3H, C**8**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.4 (C=O), 139.4 (Ar), 136.5 (Ar), 129.0 (Ar), 128.9 (Ar), 128.3 (Ar), 127.0 (Ar), 119.8 (Ar), 67.3 (C**5**), 62.2 (C**2'**), 59.6 (C**2**), 54.6 (C**6**), 28.1 (C**4**), 22.9 (C**3**); HRMS: (ES^+) Calculated for $C_{19}H_{22}ClN_2O_2$: 345.1291. Found $[M+H]^+$: 345.1370.

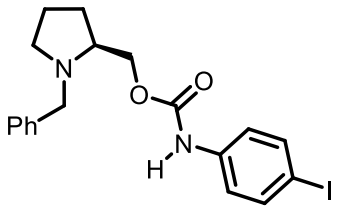
4.4.2. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl (3,5-dichlorophenyl) carbamate (3.27.2)



Following general procedure B; Yellow oil; Yield: 83%; *v* max/ cm^{-1} : 3381 (NH), 1720 (C=O) and 1206 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30 (m, 7H, 7 x Ar), 7.04 (s, 1H, Ar), 6.64 (s, 1H, NH), 4.15 (m, 2H, C**6**-CH_2), 4.05 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 3.47 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 2.99 (m, 1H, C**2**-H), 2.86 (m, 1H, C**5**-H),

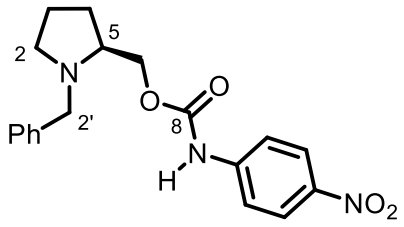
2.30 (m, 1H, C**2**-H), 1.99 (m, 1H, C**4**-H), 1.76 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.0 (C=O), 139.8 (Ar), 139.8 (Ar), 139.4 (Ar), 135.3 (Ar), 128.9 (Ar), 128.3 (Ar), 127.0 (Ar), 123.3 (Ar), 116.7 (Ar), 67.5 (C**5**), 62.2 (C**2'**), 59.6 (C**2**), 54.7 (C**6**), 28.1 (C**4**), 23.0 (C**3**); HRMS (ES^+) calculated for $C_{19}H_{21}Cl_2N_2O_2$: 379.0902. Found $[M+H]^+$: 379.0980.

4.4.3. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl (4-iodophenyl) carbamate (3.27.3)



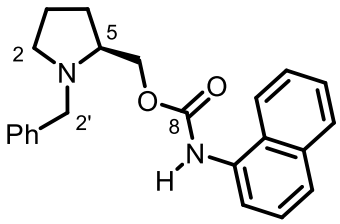
Following general procedure B; Yellow oil; yield: 79 %; *v* max/ cm^{-1}: 3351 (NH), 1712 (C=O) and 1222 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 (m, 2H, 2 x Ar), 7.30 (m, 5H, 5 Ar), 7.18 (d, 2H, 2 x Ar), 6.59 (s, 1H, NH), 4.20 (dd, *J* = 11.0, 5.0 Hz, 1H, C**6**-H), 4.15 (m, 1H, C**6**-H), 4.01 (d, *J* = 13.0 Hz, 2H, C**2'**-H), 3.45 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 2.98 (m, 1H, C**2**-H), 2.86 (m, 1H, C**5**-H), 2.30 (app. q, 1H, C**2**-H), 1.98 (m, 1H, C**4**-H), 1.73 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.9 (C=O), 128.9 (Ar), 128.3 (Ar), 127.0 (Ar), 62.2 (C**2'**), 59.6 (C**2**), 54.6 (C**6**), 28.1 (C**4**), 22.9 (C**3**); HRMS (ES^+) calculated for $C_{19}H_{22}IN_2O_2$: 437.0647. Found $[M+H]^+$: 437.0726.

4.4.4. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl (4-nitrophenyl) carbamate (3.27.4)



Following general procedure B; Bright yellow oil; yield: 82 %; *v* max/ cm^{-1}: 3321 (NH), 1697 (C=O) and 1182 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.73 (s, 1H, NH), 8.20 (d, *J* = 9.0 Hz, 2H, 2 x Ar), 7.90 (d, *J* = 7.5 Hz, 2H, 2 x Ar), 7.35 (d, *J* = 7.5 Hz, 1H, Ar), 7.28 (m, 1H, Ar), 7.19 (t, *J* = 1.5 Hz, 2H, 2 x Ar), 5.11 (dd, *J* = 13.0, 8.5 Hz, 1H, C**6**-H), 4.70 (dd, *J* = 12.5, 3.5 Hz, 1H, C**6**-H), 4.48 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.02 (d, *J* = 12.0 Hz, 1H, C**2'**-H), 3.38 (dd, *J* = 20.0, 10.5 Hz, 1H, C**2**-H), 3.19 (m, 1H, C**5**-H), 2.25 (m, 1H, C**2**-H), 2.14 (m, 1H, C**4**-H), 2.03 (m, 2H, C**4**-H and C**3**-H), 1.84 (m, 1H, C**3**-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.8 (C=O), 143.9 (Ar), 143.0 (Ar), 139.4 (Ar), 128.9 (Ar), 128.3 (Ar), 127.0 (Ar), 125.2 (Ar), 117.7 (Ar), 67.7 (C**5**), 62.2 (C**2'**), 59.6 (C**2**), 54.7 (C**6**), 28.1 (C**4**), 23.0 (C**3**); HRMS: (ES^+) Calculated for $C_{19}H_{22}N_3O_4$: 356.1532. Found $[M+H]^+$: 356.1610.

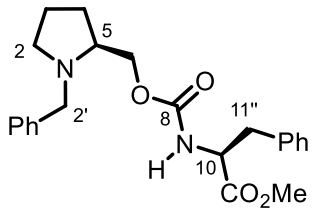
4.4.5. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl naphthalen-1-ylcarbamate (3.27.5)



Following general procedure B; Yellow oil; yield: 59%; *v* max/ cm^{-1} : 3356 (NH), 1718 (C=O) and 689 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (m, 3H, 3 x Ar), 7.67 (d, *J* = 12.5 Hz, 2H, 2 x Ar), 7.53 (m, 3H, 3 x Ar), 7.32 (m, 5H, 5 x Ar), 6.93 (s, 1H, NH), 4.25 (m, 2H, C**6**-CH_2), 4.09 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 3.45 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 2.96 (m, 2H, C**2**-H and C**5**-H), 2.29 (m, 1H, C**2**-H), 2.00 (m, 1H, C**4**-H),

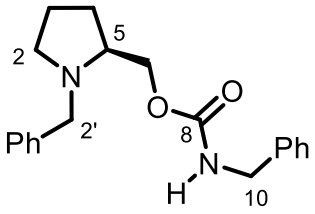
1.72 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.1 (C=O), 139.5 (Ar), 134.1 (Ar), 132.6 (Ar), 128.9 (Ar), 128.7 (Ar), 128.2 (Ar), 126.9 (Ar), 126.2 (Ar), 126.0 (Ar), 125.8 (Ar), 120.5 (Ar), 119.5 (Ar), 67.7 (C**5**), 62.24 (C**2'**), 59.5 (C**2**), 54.5 (C**6**), 28.3 (C**4**), 23.0 (C**3**); HRMS: (ESI^+) Calculated for $C_{23}H_{29}N_2O_4$: 397.2049. Found $[M+H]^+$: 397.2127.

4.4.6. Preparation of methyl ((((*(S)*-1-benzylpyrrolidin-2-yl)methoxy)carbonyl)-*L*-phenylalaninate (3.27.6)



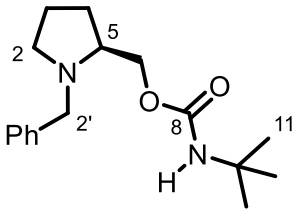
Following general procedure B; Yellow oil; yield: 58%; *v* max/ cm^{-1} : 3356 (NH), 1718 (C=O), 1697 (C=O) and 689 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25 (m, 10H, 10 x Ar), 7.10 (s, 1H, NH), 5.16 (d, *J* = 7.0 Hz, 2H, C**11''**-H), 4.66 (d, *J* = 7.0 Hz, 2H, C**6**-H_2), 4.05 (m, 2H, C**2'**-H), 3.71 (s, 3H, Me), 3.38 (m, 1H, C**2'**-H), 3.11 (m, 2H, C**2**-H and C**2**-H), 2.92 (m, 1H, C**2**-H), 2.76 (m, 1H, C**5**-H), 2.23 (q, 1H, C**2**-H), 1.93 (m, 1H, C**4**-H), 1.70 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.0 (C=O), 155.9 (C=O), 139.5 (Ar), 135.8 (Ar), 129.3 (Ar), 128.9 (Ar), 128.6 (Ar), 128.2 (Ar), 127.1 (Ar), 126.9 (Ar), 62.2 (C**5**), 62.1 (C**2'**), 59.4 (C**2**), 54.8 (C**6**), 54.4 (C**11''**), 52.3 (**Me**), 38.3 (C**10**), 22.9 (C**4**), 21.0 (C**3**); HRMS: (ESI^+) Calculated for $C_{23}H_{29}N_2O_4$: 397.2049. Found $[M+H]^+$: 397.2127.

4.4.7. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl benzylcarbamate (3.27.7)



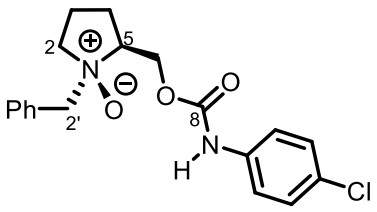
Following general procedure B; Yellow oil; yield: 82%; *v* max/ cm^{-1} : 3402 (NH), 1706 (C=O) and 698 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 (m, 10H, 10 x Ar), 5.00 (s, 1H), 4.38 (d, *J* = 6.0 Hz, 2H, C**10**-CH_2), 4.17 (dd, , *J* = 10.5, 5.0 Hz, 2H, C**6**-CH_2), 4.11 (m, 1H, C**2'**-H), 3.40 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 2.94 (m, 1H, C**2**-H), 2.81 (m, 1H, C**5**-H), 2.24 (app. q, 1H, C**2**-H), 1.93 (m, 1H, C**4**-H), 1.73 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.7 (C=O), 139.5 (Ar), 138.5 (Ar), 128.9 (Ar), 128.7 (Ar), 128.2 (Ar), 127.6 (Ar), 127.5 (Ar), 126.9 (Ar), 67.4 (C**5**), 62.3 (C**2'**), 59.5 (C**2**), 54.5 (C**6**), 45.1 (C**10**), 28.2 (C**4**), 22.9 (C**3**); HRMS (ES^+) calculated for $C_{20}H_{25}N_2O_2$: 325.1916. Found $[M+H]^+$: 325.1917.

4.4.8. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl *tert*-butylcarbamate (3.27.8)



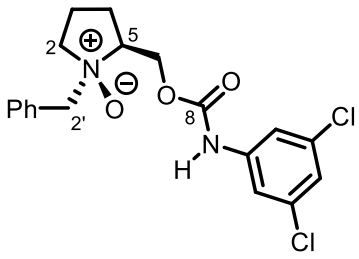
Following general procedure B; Yellow oil; yield: 98%; *v* max/ cm^{-1} : 3369 (NH), 1687 (C=O) and 706 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (m, 5H, 5 x Ar), 4.64 (s, 1H, NH), 4.15 (dd, *J* = 15.5, 8.5 Hz, 1H, C**6**-H), 4.09 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 3.99 (dd, *J* = 11.0, 6.0 Hz, 1H, C**6**-H), 3.39 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 2.94 (m, 1H, C**2**-H), 2.77 (m, 1H, C**5**-H), 2.26 (app. q, *J* = 8.5 Hz, 1H, C**2**-H), 1.94 (m, 1H, C**4**-H), 1.72 (m, 3H, C**4**-H and C**3**-CH_2), 1.32 (s, 9H 3 x C**11**-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0 (C=O), 139.5 (Ar), 129.0 (Ar), 128.2 (Ar), 126.8 (Ar), 66.4 (C**5**), 62.4 (C**2'**), 59.4 (C**2**), 54.4 (C**6**), 50.3 (C**10**), 29.0 (C**4**), 22.8 (C**3**), 21.0 (C**11**); HRMS: (ESI^+) Calculated for $C_{17}H_{27}N_2O_2$: 291.1994. Found $[M+H]^+$: 291.2073.

4.4.9. Preparation of (2S)-1-benzyl-2-((((4-chlorophenyl)carbamoyl)oxy) methyl)pyrrolidine 1-oxide (3.28.1)



Following general procedure C/D; White solid; yield: general procedure A2, 85%, general procedure A3, 72%; M.p. 108-111 ºC; v max/ cm^{-1} : (solid) 3028 (C-H), 1718 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ 7.66 (m, 2H, 2 x Ar), 7.48 (m, 5H, 5 x Ar), 7.32 (m, 2H, 2 x Ar), 4.74 (m, 2H, C**2'**-H and C**6**-H), 4.57 (dd, *J* = 12.5, 3.0 Hz, 1H, C**6**-H), 4.52 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.04 (m, 1H, C**5**-H), 3.62 (m, 1H, C**2**-H), 3.06 (m, 1H, C**2**-H), 2.18 (m, 2H, C**3**-H and C**4**-H), 1.95 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 153.5 (C**8**), 137.6 (Ar), 132.2 Ar), 131.0 (Ar), 129.2 (Ar), 128.4 (Ar), 128.2 (Ar), 127.7 (Ar), 119.8 (Ar), 74.2 (C**5**), 70.1 (C**2'**), 66.1 (C**2**), 61.1 (C**6**), 24.6 (C**4**), 18.8 (C**3**); HRMS: (ES^+) Calculated for $C_{19}H_{22}ClN_2O_3$: 361.1241. Found $[M+H]^+$: 361.1320;

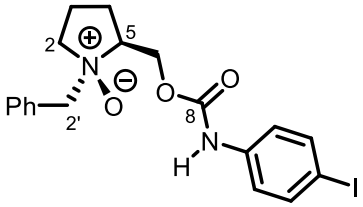
4.4.10. Preparation of (2*S*)-1-benzyl-2-((((3,5-dichlorophenyl)carbamoyl)oxy) methyl)pyrrolidine 1-oxide (3.28.2)



Following general procedure C/D; White solid; yield: general procedure A2, 88%, general procedure A3, 85%; M.p. 120-122 ºC; v max/ cm^{-1} : (solid) 2980 (C-H), 1722 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.66 (m, 2H, Ar), 7.51 (m, 2H, Ar), 7.45 (m, 3H, Ar), 7.05 (m, 1H, Ar), 4.78 (dd, *J* = 12.5, 4.0 Hz, 1H, C**6**-H), 4.72 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.55 (m, 2H, C**6**-H and C**2'**-H), 4.05 (m, 1H, C**5**-H), 3.65 (m, 1H, C**2**-H), 3.05 (m, 1H, C**2**-H), 2.22 (m, 1H, C**4**-H), 2.14 (m, 1H, C**3**-H), 1.95 (m, 2H,

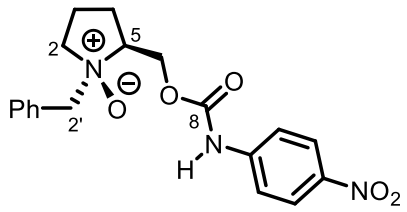
C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 153.1 (C**8**), 141.2 (Ar), 134.9 (Ar), 132.2 (Ar), 130.9 (Ar), 129.2 (Ar), 128.2 (Ar), 122.1 (Ar), 116.4 (Ar), 74.2 (C**5**), 70.1 (C**2'**), 66.1 (C**2**), 61.4 (C**6**), 24.7 (C**4**), 18.9 (C**3**); HRMS (CI^+) calculated for $C_{19}H_{21}Cl_2N_2O_3$: 395.0851. Found $[M+H]^+$: 395.0930

4.4.11. Preparation of (2S)-1-benzyl-2-((((4-iodophenyl)carbamoyl)oxy) methyl)pyrrolidine 1-oxide (3.28.3)



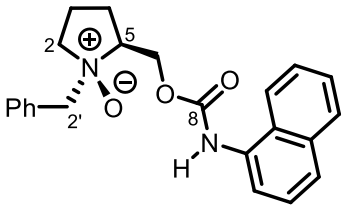
Following general procedure C/D; White foam; yield: general procedure A2, 75%, general procedure A3, 78%; M.p. 110-113 ºC; *v* max/ cm^{-1}: (solid) 2961 (C-H), 1721 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.63 (m, 4H, Ar), 7.44 (m, 3H, Ar), 7.30 (m, 2H, Ar), 4.76 (m, 2H, C**2'**-H and C**6**-H), 4.56 (dd, *J* = 12.5, 3.0 Hz, 1H, C**6**-H), 4.52 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.01 (m, 1H, C**5**-H), 3.59 (dt, *J* = 11.0, 8.5 Hz, 1H, C**2**-H), 3.04 (m, 1H, C**2**-H), 2.15 (m, 2H, C**3**-H and C**4**-H), 1.96 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 153.4 (C**8**), 138.7 (C Ar), 137.5 (C Ar), 132.2 (C Ar), 130.94 (Ar), 129.2 (Ar), 129.1 (Ar), 121.0 (Ar), 128.2 (Ar), 120.4 (Ar), 72.3 (C**5**), 70.1 (C**2'**), 66.0 (C**2**), 61.1 (C**6**), 24.6 (C**4**), 18.8 (C**3**); HRMS (ES^+) calculated for $C_{19}H_{22}IN_2O_3$: 453.0600. Found $[M+H]^+$: 453.0675.

4.4.12. Preparation of (2*S*)-1-benzyl-2-((((4-nitrophenyl)carbamoyl)oxy) methyl)pyrrolidine 1-oxide (3.28.4)



Following general procedure C/D; Bright yellow foam; yield: general procedure A2, 95%, general procedure A3, 95%. M.p. 92-97 ºC; *v* max/ cm^{-1} : (solid) 2970 (C-H), 1721 (s, C=O), 1496 (N-O), 1328 (N-O); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.16 (m, 2H, 2 x Ar), 7.73 (m, 2H, 2 x Ar), 7.67 (m, 2H, 2 x Ar), 7.46 (m, 3H, 3 x Ar), 4.82 (dd, *J* = 12.5, 8.0 Hz, 1H, C**6**-H), 4.73 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.61 (dd, *J* = 12.5, 3.0 Hz, 1H, C**6**-H), 4.53 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.06 (m, 1H, C**5**-H), 3.63 (m, 1H, C**2**-H), 3.05 (m, 1H, C**2**-H), 2.26 (m, 2H, C**3**-H and C**4**-H), 2.00 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 153.1 (C**8**), 145.2 (Ar), 142.6 (Ar), 132.2 (Ar), 131.1 (Ar), 129.1 (Ar), 128.1 (Ar), 124.5 (Ar), 117.6 (Ar), 74.2 (C**5**), 70.2 (C**2'**), 66.1 (C**2**), 61.5 (C**6**), 24.6 (C**4**), 18.9 (C**3**); HRMS: (ESI^+) Calculated for $C_{19}H_{22}N_3O_5$: 372.1481. Found $[M+H]^+$: 372.1560.

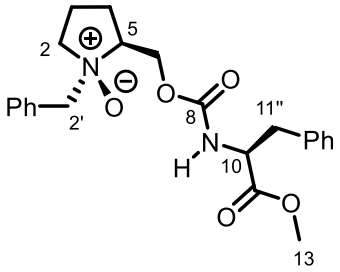
4.4.13. Preparation of (2*S*)-1-benzyl-2-(((naphthalen-1-ylcarbamoyl)oxy)methyl) pyrrolidine 1-oxide (3.28.5)



Following general procedure C/D; White foam; yield: general procedure A2, 80%, general procedure A3, 92%; M.p. 98-104 ºC; v max/ cm^{-1} : (solid) 2957 (C-H), 1712 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.11 (d, *J* = 5.0 Hz, 1H, Ar), 7.87 (m, 1H, Ar), 7.70 (m, 2H, 2 x Ar), 7.53 (d, *J* = 5.0 Hz, 1H, Ar), 7.48 (broad m, 6H, 6 x Ar),

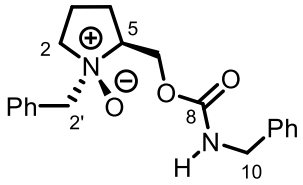
4.74 (broad m, 2H, C**2'**-H and C**6**-H), 4.53 (broad m, 2H, C**2'**-H and C**6**-H), 3.86 (m, 1H, C**5**-H), 3.40 (m, 1H, C**2**-H), 3.00 (m, 1H, C**2**-H), 2.14 (broad m, 2H, C**3**-H and C**4**-H), 1.77 (broad m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 155.3 (C**8**), 134.3 (Ar), 133.1 (Ar), 132.2 (Ar), 130.9 (Ar), 129.1 (Ar), 128.1 (Ar), 128.0 (Ar), 125.9 (Ar), 125.9 (Ar), 125.3 (Ar), 121.9 (Ar), 74.0 (C**5**), 70.0 (C**2'**), 66.0 (C**2**), 61.3 (C**6**), 24.5 (C**4**), 18.8 (C**3**); HRMS: (ESI$^+$) Calculated for $C_{23}H_{25}N_2O_3$: 377.1870. Found $[M+H]^+$: 377.1865.

4.4.14. Preparation of (2S)-1-benzyl-2-(((((*(S)*-1-methoxy-1-oxo-3-phenylpropan-2-yl)carbamoyl)oxy)methyl)pyrrolidine 1-oxide (3.28.6)



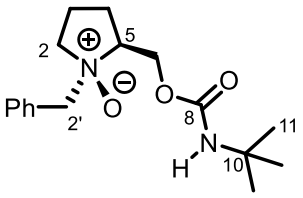
Following general procedure C; Yellow viscous oil; yield: 89%; *v* max/ cm^{-1} : (solid) 2980 (C-H), 1720 (s, C=O), 1707 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.60 (m, 2H, 2 x Ar), 7.47 (m, 3H, 2 x Ar), 7.24 (m, 5H, 5 x Ar), 4.42 (m, 5H, C**11'**-CH_2 and C**2'**-CH_2 and C**6**-H), 3.85 (m, 1H, C**5**-H), 3.72 (s, 3H, C1**3**-CH_3), 3.56 (m, 1H, C**2**-H), 3.19 (m, 1H, C**2**-H), 2.07 (m, 2H, C**3**-H and C**4**-H), 1.81 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 172.4 (C**11**), 156.2 (C**8**), 132.3 (Ar), 129.1 (Ar), 128.8 (Ar), 128.2 (Ar), 128.1 (Ar), 128.1 (Ar), 126.5 (Ar), 74.2 (C**5**), 70.1 (C**2'**), 66.0 (C**2**), 61.0 (C**6**), 55.6 (C**10**), 51.3 (C**13**), 37.1 (C**11''**), 24.4 (C**4**), 18.8 (C**3**); HRMS: (ESI$^+$) Calculated for $C_{23}H_{29}N_2O_5$: 413.1998. Found $[M+H]^+$: 413.2076.

4.4.15. Preparation of (2*S*)-1-benzyl-2-(((benzylcarbamoyl)oxy)methyl) pyrrolidine 1-oxide (3.28.7)



Following general procedure C/D; Colourless viscous oil; yield: general procedure A2, 95%, general procedure A3, 75%; *v* max/ cm^{-1}: (solid) 2980 (C-H), 1707 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.63 (m, 2H, 2 x Ar), 7.45 (m, 3H, 3 x Ar), 7.35 (m, 4H, 4 x Ar), 7.27 (m, 1H, Ar), 4.70 (dd, *J* = 12.0, 8.0 Hz, 1H, C**6**-H), 4.66 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.49 (m, 2H, C**6**-H and C**2'**-H), 4.35 (ABq, *J* = 8.0 Hz, 2H, C**10**-H), 3.93 (m, 1H, C**5**-H), 3.57 (m, 1H, C**2**-H), 3.03 (m, 1H, C**2**-H), 2.13 (m, 2H, C**3**-H and C**4**-H), 1.94 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 156.8 (C**8**), 139.1 (Ar), 132.2 (Ar), 131.0 (Ar), 129.1 (Ar), 128.2 (Ar), 128.1 (Ar), 127.0 (Ar), 126.8 (Ar), 73.9 (C**5**), 70.1 (C**2'**), 66.0 (C**2**), 61.0 (C**6**), 44.2 (C**10**), 24.5 (C**4**), 18.8 (C**3**); HRMS (ES^+) calculated for $C_{20}H_{25}N_2O_3$: 341.1787. Found $[M+H]^+$: 341.1865.

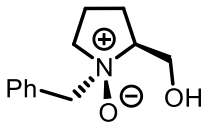
4.4.16. Preparation of (2*S*)-1-benzyl-2-(((*tert*-butylcarbamoyl)oxy)methyl) pyrrolidine 1-oxide (3.28.8)



Following general procedure C/D; White crystalline solid; yield: general procedure A2, 92%, general procedure A3, 88%; M.p. 92-95 ºC; *v* max/ cm^{-1} : (solid) 2970 (C-H), 1707 (s, C=O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.64 (m, 2H, 2 x Ar), 7.47 (m, 3H, 3 x Ar), 4.69 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 4.58 (dd, *J* = 12.5, 8.5 Hz, 1H, C**6**-H), 4.51 (d, *J* = 13.0 Hz, 1H, C**2'**-H), 4.42 (dd, *J* = 12.5, 3.0 Hz, 1H, C**6**-H), 3.91 (m, 1H, C**5**-H), 3.59 (m, 1H, C**2**-H), 3.06 (m, 1H, C**2**-H), 2.16 (m, 2H, C**3**-H and C**4**-H), 1.91 (m, 2H, C**3**-H and C**4**-H), 1.34 (s, 9H, 3 x C**11**-CH_3); ^{13}C NMR (126 MHz, CD_3OD)

δ: 154.8 (C**8**), 132.2 (Ar), 130.9 (Ar), 129.2 (Ar), 128.2 (Ar), 74.2 (C**5**), 69.9 (C**2'**), 65.9 (C**2**), 60.1 (C**6**), 49.7 (C**10**), 27.7 (C**11**), 24.5 (C**4**), 18.8 (C**3**); HRMS: (ESI^+) Calculated for $C_{23}H_{25}N_2O_3$: 377.1787. Found $[M+H]^+$: 377.1865.

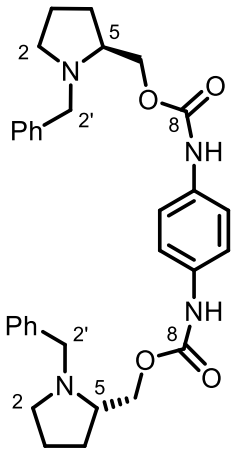
4.4.17. Preparation of *N*-benzyl-*L*-Prolinol *N*-oxide (3.29)



To a stirred solution of *N*-benzylprolinol (0.50 g, 2.61 mmol, 1.0 eq.) in DCM (26 mL) at -78 °C was added *m*-CPBA (1.2 eq.) and K_2CO_3 (1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.51 g, 95%) as a white crystalline solid.

^{1}H NMR (500 MHz, CD_3OD) δ: 7.61 (m, 2H, 2 x Ar), 7.43 (m, 3H, 3 x Ar), 4.63 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.54 (d, *J* = 12.5 Hz, 1H, C**2'**-H), 4.14 (dd, *J* = 12.5, 6.5 Hz, 1H, C**6**-H), 3.90 (dd, *J* = 12.5, 3.0 Hz, 1H, C**6**-H), 3.66 (m, 1H, C**5**-H), 3.48 (m, 1H, C**2**-H), 3.03 (m, 1H, C**2**-H), 2.07 (m, 3H, C**4**-CH_2 and C**3**-H), 1.90 (m, 1H, C**3**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 136.3 (Ar), 135.0 (Ar), 133.0 (Ar), 132.1 (Ar), 78.5 (C**5**), 73.6 (C**2'**), 69.8 (C**2**), 62.8 (C**6**), 27.9 (C**4**), 22.9 (C**3**); HRMS: (ES^+) Calculated for $C_{12}H_{18}NO_2$: 208.1323. Found $[M+H]^+$: 208.1325.

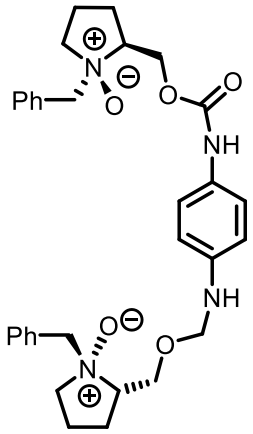
4.4.18. Preparation of ((*S*)-1-benzylpyrrolidin-2-yl)methyl (4-(((((*S*)-1-benzylpyrrolidin-2-yl)methoxy)methyl)amino)phenyl)carbamate (3.30)



To a stirred solution of *N*-benzylprolinol (0.30 g, 1.57 mmol, 1.0 eq.) in THF (15 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.07 g, 1.72 mmol, 1.1 eq.). The reaction mixture was allowed to stir at 0 °C for 30 mminutes followed by the addition of 1,4-phenyl diisocyanate (0.12 g, 0.76 mmol, 0.5 eq.). The reaction was heated to reflux and stirred for a further 18 hours. The reaction was quenched with MeOH and solvent removed *in vacuo*. The resulting residue was taken up in DCM (50 mL) and H_2O (50 mL) and extracted with DCM (2 x 50 mL), combined organic extracted dried ($MgSO_4$) and solvents removed *in vacuo*. The residue was purified by FCC (eluting with 2:8 MeOH:EtOAc) to afford title compound (0.27 g, 64%) as a yellow solid.

M.p. 78-82 ºC; *v* max/ cm^{-1} : 3400 (N-H), 1697 (C=O), 1303 (C-O) and 1217 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 10H 10 x Ar-H), 7.28 (m, 4H, 4 x Ar-H), 4.24 (dd, 2H, *J* = 11.0, 5.0 Hz, 1H, 2 x C**6**-H), 4.16 (dd, 2H, *J* = 11.0, 6.0 Hz, 1H, 2 x C**6**-H), 4.11 (d, 2H, *J* = 13.0 Hz, 1H, 2 x C**2'**-H), 3.49 (d, 2H *J* = 13.0 Hz, 1H, 2 x C**2'**-H), 3.02 (m, 2H, 2 x C**2**-H), 2.90 (m, 4H, 2 x C**2**-H and 2 x C**5**-H), 2.32 (m, 2H, 2 x C**4**-H), 2.01 (m, 2H, 2 x C**3**-H), 1.78 (m, 4H, 2 x C**2**-H and 2 x C**3**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.7 (C**8**), 139.2 (Ar), 133.6 (Ar), 129.0 (Ar), 128.3 (Ar), 127.0 (Ar), 119.5 (Ar), 67.1 (C**6**), 62.3 (C**5**), 59.5 (C**6'**), 54.6 (C**2**), 28.2 (C**4**), 22.9 (C**3**); HRMS: (ES^+) calculated for $C_{32}H_{39}N_4O_4$: 543.2971. Found $[M+H]^+$: 543.2960.

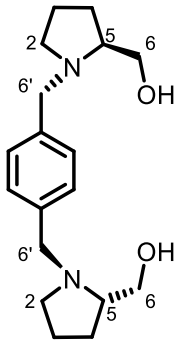
4.4.19. Preparation of (1*R*,2*S*)-1-benzyl-2-((((4-(((((1*R*,2*S*)-1-benzyl-1-oxidopyrrolidin-2-yl)methoxy)carbonyl)amino)phenyl)amino)methoxy)methyl)pyrrolidine 1-oxide (3.31)



To a stirred solution of **3.30** (0.15 g, 0.28 mmol, 1.0 eq.) in DCM (25 mL) at -78 °C was added *m*-CPBA (52 mg, 0.34 mmol, 1.2 eq.) and K_2CO_3 (58 mg, 0.42 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford the title compound (0.04 g, 34%) as yellow solid.

M.p. 148-153 ºC; *v* max/ cm^{-1} : 3316 (N-H), 1731 (C=O), 1530 (N-H), 1302 (C-O) and1217 (C-N); ^{1}H NMR (500 MHz, CD_3OD) δ 7.68-7.63 (m, 4H, 4 x Ar), 7.42 (m, 10H, 10 x Ar), 4.73 (m, 4H, 2 x C**6**-H and 2 x C**2'**-H), 4.52 (m, 4H, 2 x C**6**-H and 2 x C**2'**-H), 4.00 (apparent q, *J* = 16.0, 8.0 Hz, 2H, 2 x C**5**-H), 3.56 (apparent q, *J* = 20.0, 10.5 Hz, 2H, 2 x C**2**-H), 3.00 (m, 2H, 2 x C**2**-H), 2.15 (m, 4H, 2 x C**4**-H and 2 x C**3**-H), 1.90 (m, 4H, 2 x C**3**-H and 2 x C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 162.5 (C**8**), 132.2 (Ar), 131.0 (Ar), 129.1 (Ar), 128.2 (Ar), 74.2 (C**5**), 70.1 (C**2'**), 66.1 (C**2**), 61.0 (C**6**), 24.6 (C**4**), 18.8 (C**3**); HRMS: (ES^+) calculated for $C_{32}H_{38}N_4O_6$: 574.2870. Found $[M+H]^+$: 575.2866.

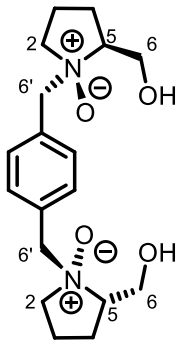
4.4.20. Preparation of ((2*S*,2′*S*)-(1,4-phenylenebis(methylene))bis(pyrrolidine-1,2-diyl))dimethanol (3.33)



To a stirred solution of *L*-prolinol (1.0 g, 9.89 mmol, 1.0 eq.) in toluene (25 mL) was added dibromo-*p*-xylene (1.30 g, 4.94 mmol, 0.5 eq.) and K_2CO_3 (2.05 g, 14.83 mmol, 1.5 eq.). The reaction was heated to reflux for 48 hours and then solvents were removed *in vacuo*. The residue was taken up in MeOH, filtered to remove solid and reduced *in vacuo* to give the title compound (1.20 g, 80%) as a yellow oil.

M.p. 65-68 ºC; *v* max/ cm^{-1} : 3305 (O-H) and 695 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, 4H, *J* = 13.5 Hz, 4 x Ar), 3.95 (d, 2H, *J* = 13.0 Hz, 2 x C**6'**-H), 3.64 (dd, 2H, *J* = 10.5, 3.5 Hz, 2 x C**6**-H), 3.43 (dd, 2H, *J* = 10.5, 1.5 Hz, 2 x C**6**-H), 3.37 (d, 2H, *J* = 13.0 Hz, 2 x C**6'**-H), 3.98 (m, 2H, 2 x C**2**-H), 2.82 (s, 2H, 2 x OH), 2.73 (m, 2H, 2 x C**5**-H), 2.30 (dd, 2H, *J* = 17.0, 9.0 Hz, 1H, 2 x C**2**-H), 1.93 (m, 2H, 2 x C**4**-H), 1.84 (m, 2H, 2 x C**3**-H), 1.70 (m, 4H, 2 x C**4**-H and 2 x C**3**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.1 (2 x C**7'**), 128.7 (4 x C**8'**), 64.2 (2 x C**5**), 61.9 (2 x C**6'**), 58.3 (2 x C**6**), 54.5 (2 x C**2**), 27.8 (2 x C**4**), 23.4 (2 x C**3**); HRMS (ES^+) calculated for $C_{18}H_{29}N_2O_2$: 3005.2224. Found $[M+H]^+$: 305.2230.

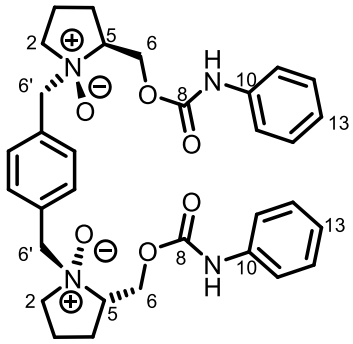
4.4.22. Preparation of (1*R*,1'*R*,2*S*,2'*S*)-1,1'-(1,4-phenylenebis(methylene))bis(2-(hydroxymethyl)pyrrolidine 1-oxide) (3.35)



To a stirred solution of bis-amine **3.33** (0.20 g, 0.66 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.27 g, 1.58 mmol, 2.4 eq.) and K_2CO_3 (0.23 g, 1.64 mmol, 2.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.14 g, 64%) as a white solid.

M.p. 172-175 ºC; *v* max/ cm^{-1} : 3358 (O-H), 1323 (C-O) and 1197 (C-N); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.72 (s, 4H, Ar), 4.70 (d, 2H, *J* = 12.5 Hz, C**6'**-H_2), 4.58 (d, 2H, *J* = 12.5 Hz, C**6'**-H_2), 4.17 (dd, 2H, *J* = 12.5, 6.5 Hz, C**6**-H_2), 3.91 (dd, 2H, *J* = 12.5, 2.5 Hz, C**6**-H_2), 3.71 (m, 2H, C**5**-H_2), 3.53 (m, 2H, C**2**-H_2), 3.04 (m, 2H, C**2**-H_2), 2.08 (m, 6H, C**4**-H_2 x 2 and C**3**-H_2), 1.90 (m, 2H, C**3**-H_2); ^{13}C NMR (101 MHz, CD_3OD) δ: 132.3 (Ar), 75.0 (C**5**), 69.2 (C**6'**), 66.0 (C**2**), 58.8 (C**6**), 24.0 (C**4**), 19.0 (C**3**); HRMS: (ES^+) calculated for $C_{18}H_{29}N_2O_4$: 337.2127. Found $[M+H]^+$: 337.2135.

4.4.23. Preparation of (1*R*,1'*R*,2*S*,2'*S*)-1,1'-(1,4-phenylenebis(methylene))bis(2-(((phenylcarbamoyl)oxy)methyl)pyrrolidine 1-oxide) (3.36)



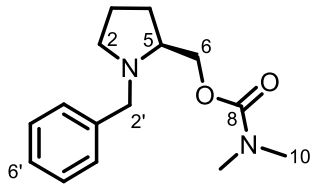
To a stirred solution of bis-alcohol **3.33** (0.30 g, 0.99 mmol, 1.0 eq.) in THF (20 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.09 g, 2.17 mmol, 2.2 eq.). The reaction mixture was allowed to stir at this temperature for 20 minutes followed by the addition of phenyl isocyanate (0.24 mL, 2.17 mmol, 2.2 eq.). The reaction was allowed to warm to ambient temperature and was allowed to stir overnight. The reaction was quenched with methanol and solvents were removed *in vacuo*. The residue was taken up in EtOAc (50 mL) and washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude bis amine which was carried forward with no further purifications.

To a stirred solution of bis-amine (0.43 g, 0.79 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.33 g, 1.90 mmol, 2.4 eq.) and K_2CO_3 (0.27 g, 1.98 mmol, 2.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.42 g, 92%) as a pale yellow solid.

M.p. 124-128 ºC; *v* max/ cm^{-1} : 3387 (N-H), 1702 (C=O), 1283 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 7.71 (s, 4H, 4 x C**4'**-H), 7.48 (d, 4H, *J* = 8.0 Hz, 4 x C**11**-H), 7.29 (t, 4H, *J* = 8.0 Hz, 4 x C**12**-H), 7.04 (t, 2H, *J* = 7.5 Hz, 2 x C**13**-H), 4.73 (m, 2H, 2 x C**6**-H and 2 x C**2'**-H), 4.54 (m, 2H, 2 x C**6**-H and 2 x C**2'**-H), 3.98 (m, 2H, 2 x C**5**-H), 3.56 (m, 2H, 2 x C**2**-H), 3.18 (m, 2H, 2 x C**2**-H), 2.12 (m, 4H, 2 x C**3**-H and 2 x C**4**-H), 2.93 (m, 4H, 2 x C**3**-H and 2 x C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 153.7 (C**8**), 138.6 (C**10**), 132.4 (C**3'**), 132.2 (C**4'**), 128.5 (C**12**), 122.9 (C**13**), 118.5 (C**11**), 74.5

(C**5**), 69.6 (C**2'**), 66.3 (C**2**), 60.9 (C**6**), 24.7 (C**4**), 18.9 (C**3**); HRMS: (ES^+) calculated for $C_{32}H_{39}N_4O_6$: 557.2870. Found $[M+H]^+$: 557.2876.

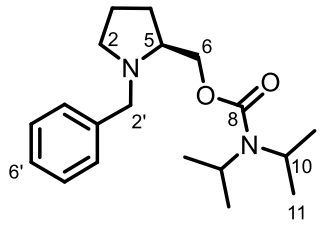
4.4.24. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl dimethylcarbamate (3.38.1)



To a stirred solution of *N*-benzyl-*L*-prolinol (0.20 g, 1.05 mmol, 1.0 eq.) in THF (8 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.05 g, 1.15 mmol, 1.1 eq.). The reaction mixture was allowed to stir at this temperature for 30 minutes followed by the addition of dimethylcarbamoyl chloride (0.11 g, 1.15 mmol, 1.1 eq.). The reaction was then stirred for 18 hours at reflux. The reaction mixture was then diluted with EtOAc (20 mL) and washed with H_2O (20 mL) saturated sodium bicarbonate solution (20 mL), brine (20 mL). The organic phase was dried ($MgSO_4$) and reduced *in vacuo*. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.18 g, 67%) as a colourless oil.

v max/ cm^{-1} : 1703 (C=O), 1162 (C-O) and 755 (C=C); ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 4H, Ar), 7.26 (m, 1H, Ar), 4.16 (m, C**6**-H and C**2'**-H), 4.08 (dd, 1H, *J* = 11.0, 6.0 Hz, C**6**-H), 3.41 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 2.93 (m, 7H, 2 x C**10**-CH_3 and C**2**-H), 2.86 (m, 1H, C**5**-H), 2.26 (m, 1H, C**2**-H), 1.97 (m, 1H, C**4**-H), 1.71 (m, 1H, C**4**-H and C**3**-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.7 (C**8**), 139.9 (C**3'**), 128.8 (Ar), 128.2 (Ar), 126.8 (C**6'**), 68.3 (C**5**), 62.4 (C**2'**), 59.5 (C**2**), 54.4 (C**6**), 36.8 (C**10**), 28.4 (C**4**), 22.9 (C**3**); HRMS: (ES^+) calculated for $C_{15}H_{23}N_2O_2$: 263.1760. Found $[M+H]^+$: 263.1772.

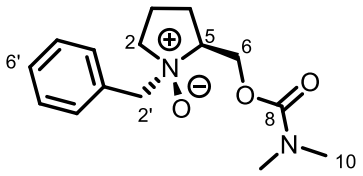
4.4.25. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl diisopropylcarbamate (3.38.2)



To a stirred solution of *N*-benzyl-*L*-prolinol (0.30 g, 1.57 mmol, 1.0 eq.) in THF (8 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.08 g, 1.88 mmol, 1.2 eq.). The reaction mixture was allowed to stir at this temperature for 30 minutes followed by the addition of di*iso*propylcarbamoyl chloride (0.28 g, 1.73 mmol, 1.1 eq.). The reaction was then stirred for 18 hours at reflux. The reaction mixture was then diluted with EtOAc (20 mL) and washed with H_2O (20 mL) saturated sodium bicarbonate solution (20 mL), brine (20 mL). The organic phase was dried ($MgSO_4$) and reduced *in vacuo*. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.37 g, 74%) as a colourless oil.

v max/ cm^{-1} : 1684 (C=O), 1154 (C-O) and 768 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 3H, Ar), 7.26 (m, 2H, Ar), 4.26 (dd, 1H, *J* = 11.0, 4.5 Hz, C**6**-H), 4.14 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 4.02 (dd, 1H, *J* = 11.0, 7.0 Hz, C**6**-H), 3.81 (br. s, 2H, 2 x C**10**-H), 3.38 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 2.92 (m, 1H, C**2**-H), 2.85 (m, 1H, C**5**-H), 2.23 (m, 1H, C**2**-H), 2.00 (m, 1H, C**4**-H), 1.73 (m, 3H, C**4**-H and C**3**-CH_3), 1.24 (d, 12H, *J* = 7.0 Hz, 4 x C**11**-CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.8 (C**8**), 139.8 (C**3'**), 128.8 (Ar), 128.2 (Ar), 126.8 (C**6'**), 67.2 (C**5**), 62.5 (C**2'**), 59.4 (C**2**), 54.3 (C**6**), 46.1 (C**10**), 28.9 (C**4**), 22.8 (C**3**), 21.2 (C**11**); HRMS: (ES^+) calculated for $C_{19}H_{31}N_2O_2$: 319.2390. Found $[M+H]^+$: 319.2395.

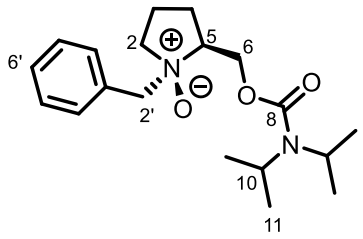
4.4.26. Preparation of (1*R*,2*S*)-1-benzyl-2-(((dimethylcarbamoyl)oxy)methyl) pyrrolidine 1-oxide (3.39.1)



To a stirred solution of tertiary amine **3.38.1** (0.25 g, 0.95 mmol, 1.0 eq.) in DCM (20 mL) at -78 °C was added *m*-CPBA (0.20 g, 1.14 mmol, 1.2 eq.) and K_2CO_3 (0.20 g, 1.43 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:4 MeOH:EtOAc) to afford title compound (0.26 g, 84%) as a colourless viscous oil.

v max/ cm^{-1} : 1642 (C=O) and 1135 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ 7.63 (m, 2H, Ar), 7.45 (m, 3H, Ar), 4.73 (dd, 1H, *J* = 12.5, 8.0 Hz, C**6**-H), 4.62 (d, 1H, *J* = 12.5 Hz, C**2'**-H), 4.51 (d, 1H, *J* = 12.5 Hz, C**2'**-H), 4.42 (dd, 1H, *J* = 12.5, 3.0 Hz, C**6**-H), 3.98 (qd, 1H, *J* = 8.0, 3.0 Hz, C**5**-H), 3.57 (m, 1H, C**2**-H), 3.00 (m, 7H, C**2**-H and 2 x C**10**-CH_3), 2.18 (m, 1H, C**4**-H), 2.12 (m, 1H, C**3**-H), 1.93 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 156.2 (C**8**), 132.1 (Ar), 130.9 (C**3'**), 129.2 (C**6'**), 128.2 (Ar), 74.4 (C**5**), 69.9 (C**6'**), 66.0 (C**2**), 61.9 (C**6**), 35.0 (C**10**), 24.8 (C**4**), 18.8 (C**3**); HRMS: (ES^+) calculated for $C_{15}H_{23}N_2O_3$: 279.1709. Found $[M+H]^+$: 279.1715.

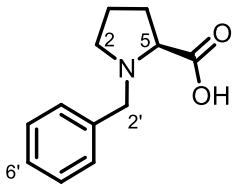
4.4.27. Preparation of (1*R*,2*S*)-1-benzyl-2-(((diisopropylcarbamoyl)oxy)methyl) pyrrolidine 1-oxide (3.39.2)



To a stirred solution of tertiary amine **3.38.2** (0.37 g, 1.16 mmol, 1.0 eq.) in DCM (20 mL) at -78 °C was added *m*-CPBA (0.24 g, 1.40 mmol, 1.2 eq.) and K_2CO_3 (0.24 g, 1.74 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:4 MeOH:EtOAc) to afford title compound (0.37 g, 95%) as a colourless viscous oil.

v max/ cm^{-1} : 1686 (C=O), 1307 (C-O) and 1065 (N-O); ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (m, 2H, Ar), 7.45 (m, 3H, Ar), 4.76 (dd, 1H, *J* = 12.5, 7.5 Hz, C**6**-H), 4.60 (d, 1H *J* = 12.5 Hz, C**2'**-H), 4.51 (d, 1H, *J* = 12.5 Hz, C**2'**-H), 4.40 (dd, 1H, *J* = 12.5, 4.0 Hz, C**6**-H), 4.13 (br. s, 2H, 2 x C**10**-H), 3.98 (m, 1H, C**5**-H), 3.56 (m, 1H, C**2**-H), 3.04 (m, 1H, C**2**-H), 2.20 (m, 1H, C**4**-H), 2.11 (m, 1H, C**3**-H), 2.93 (m, 2H, C**3**-H and C**4**-H), 1.27 (br. s, 12H, 4 x C**11**-CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 154.6 (C**8**), 132.1 (Ar), 130.8 (C**3'**), 129.3 (C**6'**), 128.2 (Ar), 74.1 (C**5**), 69.8 (C**6'**), 66.0 (C**2**), 61.3 (C**6**), 47.9 (C**10**), 25.1 (C**4**), 19.6 (C**11**), 18.8 (C**3**); HRMS: (ES^+) calculated for $C_{19}H_{31}N_2O_3$: 335.2335. Found $[M+H]^+$: 335.2338.

4.4.27. Preparation of benzyl-*L*-proline (3.14)

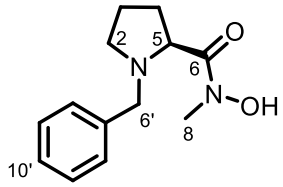


To a stirred solution of *L*-proline (5.00 g, 43.4 mmol, 1.0 eq.) in DMF (50 mL) was added BnBr (10.00 mL, 86.8 mmol, 2.0 eq.) and K_2CO_3 (14.13 g, 108.50 mmol, 2.5 eq.). The reaction mixture was allowed to stir at ambient temperature overnight. The reaction was diluted with diethyl ether (100 mL) and washed with H_2O (100 mL), saturated sodium bicarbonate solution (100 mL) and brine (100 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude dibenzyl proline intermediate.

To a stirred solution of dibenzyl proline intermediate in MeOH (150 mL) was added 10% Pd/C (0.23 g, 2.17 mmol, 0.05 eq.), and the reaction mixture was allowed to stir at rt under a H_2 atmosphere and progress followed by TLC. Upon loss of starting material the reaction was filtered through a Celite pad, and solvents were removed *in vacuo* to give crude product. The crude material was purified by FCC (eluting with 7:3 EtOAc:MeOH) to afford the title compound (6.14 g, 69%) as a white solid.

v max/ cm^{-1} : 2822 (br. OH), 1648 (C=O) and 699 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.56 (m, 2H, Ar), 7.48 (m, 3H, Ar), 4.53 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 4.31 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 4.05 (dd, 1H, *J* = 9.5, 7.0 Hz, C**5**-H), 3.55 (m, 1H, C**2**-H), 3.29 (m, 1H, C**2**-H), 2.51 (m, 1H, C**4**-H), 2.15 (m, 2H, C**3**-H and C**4**-H), 1.97 (m, 1H, C**3**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 171.1 (C=O), 130.6 (Ar), 130.3 (Ar), 129.6 (C**6'**), 128.9 (Ar), 67.9 (C**2'**), 58.1 (C**5**), 53.9 (C**2**), 28.3 (C**4**), 22.3 (C**3**).

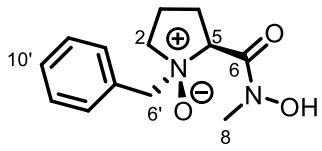
4.4.28. Preparation of *(S)*-1-benzyl-*N*-hydroxy-*N*-methylpyrrolidine-2-carboxamide (3.41)



To a stirred solution of *N*-benzyl proline (0.3 g, 1.46 mmol, 1.0 eq.) in THF (20 mL) at 0 °C was added ethyl chloroformate (0.17 mL, 1.75 mmol, 1.2 eq.) and NEt_3 (0.25 mL, 1.75 mmol, 1.2 eq.). The reaction mixture was allowed to stir for 1 hour. NHMeOH.HCl (0.12 g, 1.46 mmol, 1.0 eq.) and KOH (0.08 g, 1.46 mmol, 1.0 eq.) in MeOH (3 mL) were then added to the reaction mixture. The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was filtered and solvents were removed *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic phase was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 9:1 EtOAc:MeOH) to afford the title compound (0.15 g, 44%) as a white solid.

v max/ cm^{-1} : 2956 (O-H) and 1641 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (m, 5H, Ar), 3.89 (d, 1H, *J* = 12.5 Hz, C**6'**-H), 3.47 (d, 1H, *J* = 12.5 Hz, C**6'**-H), 3.36 (m, 1H, C**2**-H), 3.07 (s, 3H, C**8**-CH_3), 2.99 (m, 1H, C**5**-H), 2.30 (m, 1H, C**2**-H), 2.22 (m, 1H, C**3**-H), 2.13 (m, 1H, C**4**-H), 1.84 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (C**6**), 136.2 (C**7'**), 129.3 (Ar), 128.6 (Ar), 128.0 (C**10'**), 69.9 (C**5**), 59.2 (C**6'**), 53.1 (C**2**), 35.0 (C**8**), 27.7 (C**4**), 23.1 (C**3**); HRMS: (ES^+) calculated for $C_{13}H_{19}N_2O_2$: 235.1147. Found $[M+H]^+$: 235.1143.

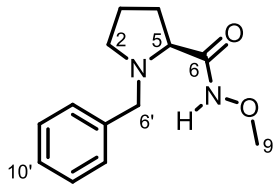
4.4.29. Preparation of (1*R*,2*S*)-1-benzyl-2-(hydroxy(methyl)carbamoyl) pyrrolidine 1-oxide (3.42)



To a stirred solution of tertiary amine **3.41** (0.45 g, 1.72 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.31 g, 1.75 mmol, 1.2 eq.) and K_2CO_3 (0.36 g, 2.58 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 2:8 MeOH:EtOAc) to afford title compound (0.0.31 g, 68%) as a white solid.

v max/ cm^{-1} : 3292 (O-H) and 1700 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (m, 2H, Ar), 7.38 (m, 3H, Ar), 4.63 (m, 1H, C**5**-H), 4.44 (s, 2H, C**6'**-CH_2), 3.35 (m, 2H, C**2**-CH_2), 3.19 (s, 3H, C**8**-CH_3), 2.89 (m, 1H, C**4**-H), 2.39 (m, 1H, C**3**-H), 2.08 (m, 1H, C**4**-H), 1.93 (m, 1H, C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5 (C**6**), 132.5 (Ar), 130.3 (C**10'**), 129.1 (C**7'**), 128.8 (Ar), 74.3 (C**5**), 70.7 (C**6'**), 65.7 (C**8**), 36.3 (C**2**), 22.9 (C**4**), 20.4 (C**3**); HRMS: (ES^+) calculated for $C_{13}H_{19}N_2O_3$: 251.1400. Found $[M+H]^+$: 251.1402.

4.4.30. Preparation of *(S)*-1-benzyl-*N*-methoxypyrrolidine-2-carboxamide (3.43)

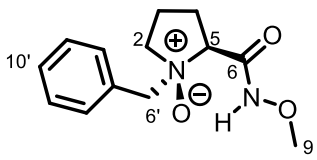


To a stirred solution of *N*-benzyl proline (0.3 g, 1.46 mmol, 1.0 eq.) in THF (20 mL) at 0 °C was added ethyl chloroformate (0.17 mL, 1.75 mmol, 1.2 eq.) and NEt_3 (0.25 mL, 1.75 mmol, 1.2 eq.). The reaction mixture was allowed to stir for 1 hour. $NH_2OMe.HCl$ (0.12 g, 1.46 mmol, 1.0 eq.) and KOH (0.08 g, 1.46 mmol, 1.0 eq.) in MeOH (3 mL) were then added to the reaction mixture. The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction mixture was filtered

and solvents were removed *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic phase was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:2 EtOAc:hexanes) to afford the title compound (0.22 g, 64%) as a yellow oil.

v max/ cm^{-1} : 3192 (N-H) and 1688 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (br. s, 1H, NH), 7.40 – 7.23 (m, 5H, Ar), 3.83 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.65 (s, 3H, C**9**-CH_3), 3.56 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.31 (dd, 1H, *J* = 10.5, 4.5 Hz, C**2**-H), 3.11 – 3.01 (m, 1H, C**5**-H), 2.38 (td, 1H, *J* = 10.0, 6.5 Hz, C**2**-H), 2.23 (m, 2H, C**4**-H), 1.97 (m, 1H, C**3**-H), 1.75 (m, 2H, C**3**-H and C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4 (C**6**), 138.3 (C**7'**), 128.8 (Ar), 128.6 (Ar), 127.5 (C**10'**), 66.6 (C**5**), 64.3 (C**9**), 60.2 (C**6'**), 54.3 (C**2**), 30.7 (C**3**), 24.2 (C**4**); HRMS: (ES^+) calculated for $C_{13}H_{19}N_2O_2$: 235.1147. Found $[M+H]^+$: 235.1148.

4.4.31. Preparation of (1*R*,2*S*)-1-benzyl-2-(methoxycarbamoyl)pyrrolidine 1-oxide (3.44)

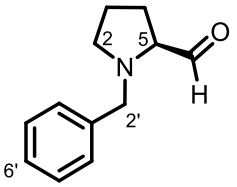


To a stirred solution of tertiary amine **3.43** (0.13 g, 0.55 mmol, 1.0 eq.) in DCM (15 mL) at -78 °C was added *m*-CPBA (0.10 g, 0.42 mmol, 1.2 eq.) and K_2CO_3 (0.12 g, 0.60 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 2:8 MeOH:EtOAc) to afford title compound (0.10 g, 72%) as a white solid.

v max/ cm^{-1} : 3202 (N-H) and 1699(C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (m, 5H, Ar), 4.61 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 4.46 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.82 (s, 3H, C**9**-CH_3), 3.77 (m, 1H, C**5**-H), 3.29 (m, 2H, C**2**-CH_2), 2.58 (m, 1H, C**4**-H), 2.32 (m, 1H, C**3**-H), 1.94 (m, 2H, C**4**-H and C**3**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4

(C**6**), 132.3 (Ar), 130.3 (C**10'**), 129.1 (Ar), 128.8 (C**7'**), 69.2 (C**5**), 64.2 (C**6'**), 62.2 (C**8**), 49.7 (C**2**), 26.6 (C**4**), 20.0 (C**3**); HRMS: (ES^+) calculated for $C_{13}H_{19}N_2O_3$: 251.1396. Found $[M+H]^+$: 251.1402.

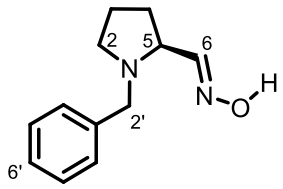
4.4.32. Preparation of *(S)*-1-benzylpyrrolidine-2-carbaldehyde (3.46)



To a stirred solution of DMSO (0.28 mL, 3.93 mmol, 3.0 eq.) in DCM (2 mL) at -78 °C was added $(COCl)_2$ (0.17 mL, 1.96 mmol, 1.5 eq.) in DCM (2 mL) dropwise. The reaction mixture was allowed to stir at this temperature for 45 minutes followed by dropwise addition of *N*-benzyl prolinol (0.25 g, 1.31 mmol, 1.0 eq.) in DCM (5 mL) dropwise. The reaction mixture was allowed to stir at -78 °C for s further 20 minutes. Triethylamine (0.73 mL, 5.24 mmol, 4.0 eq.) was the added dropwise and the reaction mixture was allowed to stir at 0 °C for 2 hours. After this time the reaction was diluted with DCM (50 mL) and washed with H_2O (50 mL) saturated sodium bicarbonate solution (50 mL), brine (50 mL). The organic phase was dried ($MgSO_4$) and reduced *in vacuo* in an ice filled water bath to give the title compound (0.25 g, 100%) as a pale yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (d, 1H, *J* = 4.0 Hz, CHO), 7.33 (m, 4H, Ar), 3.78 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.69 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.14 (m, 1H, C**5**-H), 3.01 (m, 1H, C**2**-H), 2.43 (m, 1H, C**2**-H), 2.03 (m, 1H C**4**-H), 1.88 (m, 3H, C**4**-H and C**3**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.9 (C=O), 138.4 (C**3'**), 129.2 (Ar), 128.4 (Ar), 127.4 (C**6'**), 71.7 (C**5**), 59.5 (C**2'**), 54.3 (C**2**), 26.6 (C**4**), 23.8 (C**3**);

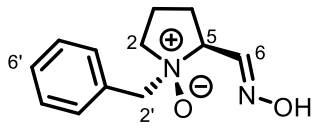
4.4.33. Preparation of(*S*,*E*)-1-benzylpyrrolidine-2-carbaldehyde oxime (3.47)



To a solution of *N*-benzyl prolinal (0.30 g, 1.59 mmol, 1.0 eq.) in THF (15mL) was added $NH_2OH.HCl$ (0.28 g, 3.98 mmol, 2.5 eq.) and NEt_3 (0.55 mL, 3.98 mmol, 2.5 eq.). The reaction mixture was allowed to stir overnight at ambient temperature. Solvents were removed *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic phase was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.25 g, 78%) as a colourless oil.

v max/ cm^{-1} : 3345 (O-H), 679 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (br. s, 1H, -OH), 7.35 (m, 4H, 3 x Ar and C**6**-H), 7.26 (m, 2H, 2 x Ar), 4.01 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.32 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.12 (m, 1H, C**5**-H), 2.99 (m, 1H, C**2**-H), 2.25 (m, 1H, C**2**-H), 2.05 (m, 1H, C**3**-H), 1.92 – 1.73 (m, 3H, C**3**-H and C**4**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.0 (C**6**), 138.6 (C**3'**), 129.1 (Ar), 128.2 (Ar), 127.0 (C**6'**), 63.0 (C**5**), 58.1 (C**2'**), 53.2 (C**2**), 29.4 (C**3**), 22.6 (C**4**); HRMS: (CI^+) calculated for $C_{12}H_{17}N_2O$: 205.1341. Found $[M+H]^+$: 205.1345.

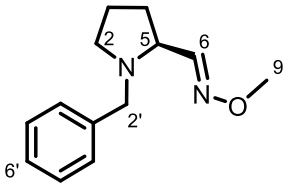
4.4.34. Preparation of (1*R*,2*S*)-1-benzyl-2-((*E*)-(hydroxyimino)methyl) pyrrolidine 1-oxide (3.48)



To a stirred solution of tertiary amine **3.47** (0.10 g, 0.50 mmol, 1.0 eq.) in DCM (10 mL) at -78 °C was added *m*-CPBA (0.10 g, 0.59 mmol, 1.2 eq.) and K_2CO_3 (0.10 g, 0.75 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:4 MeOH:EtOAc) to afford title compound (0.68 g, 62%) as a colourless viscous oil.

v max/ cm^{-1} :3366 (O-H), 706 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (m, 2H, Ar), 7.57 (d, 1H, *J* = 7.5 Hz, C**6**-H), 7.45 (m, 3H, Ar), 4.45 (m, 1H, C**2'**-H), 4.23 (m, 1H, C**5**-H), 3.55 (m, 1H, C**2**-H), 3.18 (m, 1H, C**2**-H), 2.34 (m, 1H, C**4**-H), 2.22 (m, 1H, C**4**-H and C**3**-H), 2.00 (m, 1H, C**3**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 145.1 (C**6**), 132.4 (Ar), 130.1 (C**3'**), 129.4 (C**6'**), 128.3 (Ar), 73.3 (C**5**), 68.5 (C**2'**), 64.5 (C**2**), 25.6 (C**4**), 19.5 (C**3**); HRMS: (CI^+) calculated for $C_{12}H_{17}N_2O_2$: 221.1290. Found $[M+H]^+$: 221.1290.

4.4.35. Preparation of (*S*,*E*)-1-benzylpyrrolidine-2-carbaldehyde *O*-methyl oxime (3.49)

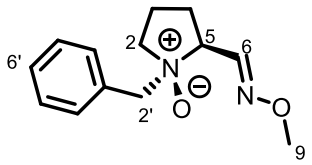


To a solution of *N*-benzyl prolinal (0.30 g, 1.59 mmol, 1.0 eq.) in THF (15mL) was added $NH_2OMe.HCl$ (0.33 g, 3.98 mmol, 2.5 eq.) and NEt_3 (0.55 mL, 3.98 mmol, 2.5 eq.). The reaction mixture was allowed to stir overnight at ambient temperature. Solvents were removed *in vacuo* and the residue was taken up in EtOAc (50 mL). The

organic phase was washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.26 g, 76%) as a colourless oil.

v max/ cm^{-1} : 1598 (C=N), 1040 (N-O) and 727 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 4H, 3 x Ar and C**6**-H), 7.26 (m, 2H, 2 x Ar), 4.00 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.87 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.31 (d, 1H, *J* = 13.0 Hz, C**2'**-H), 3.10 (m, 1H, C**5**-H), 2.99 (m, 1H, C**2**-H), 2.23 (m, 1H, C**2**-H), 2.05 (m, 1H, C**3**-H), 1.90 – 1.73 (m, 3H, C**3**-H and C**4**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7 (C**6**), 139.0 (C**3'**), 128.9 (Ar), 128.2 (Ar), 126.9 (C**6'**), 63.0 (C**5**), 61.5 (C**9**), 58.2 (C**2'**), 53.3 (C**2**), 29.6 (C**3**), 22.7 (C**4**); HRMS (ES^+) calculated for $C_{13}H_{19}N_2O$: 219.1497. Found $[M+H]^+$: 219.1503

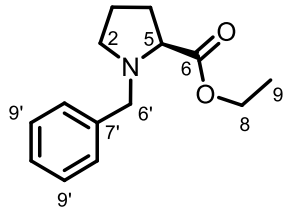
4.4.36. Preparation of (1*R*,2*S*)-1-benzyl-2-((*E*)-(methoxyimino)methyl) pyrrolidine 1-oxide (3.50)



To a stirred solution of tertiary amine **3.49** (0.10 g, 0.46 mmol, 1.0 eq.) in DCM (10 mL) at -78 °C was added *m*-CPBA (0.09 g, 0.55 mmol, 1.2 eq.) and K_2CO_3 (0.10 g, 0.69 mmol, 1.5 eq.). The reaction was allowed to warm to ambient temperature and stirred overnight. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:4 MeOH:EtOAc) to afford title compound (0.85 g, 79%) as a colourless viscous oil.

v max/ cm^{-1} : 1033 (N-O) and 716 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (d, 2H, *J* = 6.5 Hz, Ar), 7.57 (d, 1H, *J* = 7.5 Hz, C**6**-H), 7.44 (m, 3H, Ar), 4.46 (m, 2H, C**2'**-CH_2), 4.23 (m, 1H, C**5**-H), 3.95 (s, 3H, C**9**-CH_3), 3.57 (m, C**2**-H), 3.17 (m, 1H, C**2**-H), 2.34 (m, 1H, C**4**-H), 2.24 (m, 2H, C**4**-H and C**3**-H), 2.01 (m, 1H, C**3**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 145.5 (C**6**), 132.4 (Ar), 130.1 (C**3'**), 129.4 (C**6'**), 128.31 (Ar), 73.0 (C**5**), 68.8 (C**2'**), 64.9 (C**2**), 61.2 (C**9**), 25.7 (C**4**), 19.6 (C**3**); HRMS (ES^+) calculated for $C_{13}H_{19}N_2O_2$: 235.1447. Found $[M+H]^+$: 235.1450.

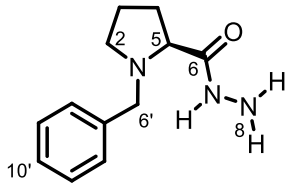
4.4.37. Preparation of *N*-benzyl proline ethyl ester (3.51)



To a stirred solution of proline (3.00 g, 26.06 mmol, 1.0 eq.) in ethanol (50 mL) was added $SOCl_2$ (1.89 mL, 26.06 mmol, 1.0 eq.) at 0 °C. The reaction mixture was heated to reflux for 4 hours and then reduced to a white residue. The residue was triturated with ether, then taken up in acetone (100 mL). BnBr (4.64 mL, 39.09 mmol, 1.5 eq.) and K_2CO_3 (7.20 g, 52.12 mmol, 2.0 eq.) were added in portions and the reaction mixture was allowed to stir overnight at ambient temperature. The reaction mixture was filtered and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:19 to 1:9 EtOAc:hexanes) to afford the title compound (5.40 g, 89%) as a colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 5H, Ar), 4.15 (qd, 1H, *J* = 7.0, 3.0 Hz, C**8**-H_2), 3.94 (d, 1H, *J* = 13.0 Hz, C**6**-H), 3.58 (d, 1H, *J* = 13.0 Hz, C**6**-H), 3.26 (dd, 1H, *J* = 9.0, 6.5 Hz, C**5**-H), 3.06 (td, 1H, *J* = 8.5, 3.0 Hz, C**2**-H), 2.41 (app. q, 1H, C**2**-H), 2.15 (m, 1H, C**4**-H), 2.00 (m, 1H, C**4**-H), 1.92 (m, 1H, C**3**-H) 1.83 – 1.75 (m, 1H, C**3**-H), 1.27 (t, 1H, *J* = 7.1 Hz, C**9**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 174.1 (C**6**), 138.5 (C**7'**), 129.2 (Ar), 128.2 (Ar), 127.1 (C**10'**), 65.4 (C**5**), 60.5 (C**8**), 58.7 (C**6'**), 53.2 (C**2**), 29.3 (C**4**), 23.0 (C**3**), 14.3 (C**9**); HRMS (ES^+) calculated for $C_{14}H_{20}NO_2$: 234.1494. Found $[M+H]^+$: 234.1497.

4.4.38. Preparation of *(S)*-1-benzylpyrrolidine-2-carbohydrazide (3.52)

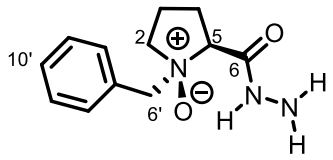


To a stirred solution of *N*-benzyl proline ethyl ester (0.50 g, 2.14 mmol, 1.0 eq.) in methanol (10 mL) was added hydrazine monohydrate (0.10 mL, 4.28 mmol, 2.0 eq.) at ambient temperature under a N_2 atmosphere. The reaction mixture was allowed to stir for a further 48 hours and then solvents removed *in vacuo* to give the crude

product. The crude material was purified by FCC (eluting with 1:9 MeOH:EtOAc) to afford the title compound (92 mg, 20%) as a pale yellow oil.

v max/ cm^{-1} : 3312 (N-H), 1735 (C=O) and 824 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.27 (br. s, 1H, NH), 7.32 (m, 5H, Ar), 3.85 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.75 (br. s, 2H, NH_2), 3.56 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.32 (dd, 1H, *J* = 10.5, 4.5 Hz, C**5**-H), 3.04 (m, 1H, C**2**-H), 2.38 (m, 1H, C**2**-H), 2.23 (m, 1H, C**4**-H), 1.92 (m, 1H, C**4**-H), 1.75 (m, 2H, C**3**-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 174.8 (C**6**), 138.4 (C**7'**), 128.8 (Ar), 128.5 (Ar), 127.4 (C**10'**), 66.5 (C**5**), 60.1 (C**6'**), 54.1 (C**2**), 30.6 (C**4**), 24.1 (C**3**). HRMS (ES^+) calculated for $C_{12}H_{18}N_3O$: 220.1450. Found $[M+H]^+$: 220.1460.

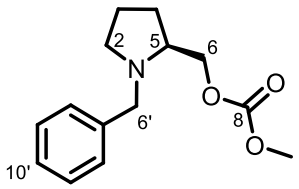
4.4.39. Preparation of (1*R*,2*S*)-1-benzyl-2-(hydrazinecarbonyl)pyrrolidine 1-oxide (3.53)



To a stirred solution of *(S)*-1-benzylpyrrolidine-2-carbohydrazide **3.52** (85 mg, 0.39 mmol, 1.0 eq.) in DCM (10 mL) at -78 °C was added *m*-CPBA (80 mg, 0.47 mmol, 1.2 eq.) and K_2CO_3 (80 mg, 0.58 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (75 mg, 82%) as a viscous oil.

v max/ cm^{-1} : 3368 and 3334 (N-H), 1703 (C=O); ^{1}H NMR (500 MHz, DMSO) δ: 11.94 (br. s, 1H, NH), 7.56 (m, 3H, Ar), 7.39 (m, 2H, Ar), 4.41 (m, 2H, C**6'**-H_2), 4.23 (s, 1H, NH), 3.88 (dd, 1H, *J* = 10.5, 8.0 Hz, C**5**-H), 3.45 (m, 1H, C**2**), 3.42 (br. s, 1H, NH), 2.90 (m, 1H, C**2**-H), 2.26 (m, 1H, C**4**-H), 2.07 (m, 2H, C**4**-H and C**3**-H), 1.82 (m, 1H C**3**-H); ^{13}C NMR (126 MHz, CH3OD) δ: 167.4 (C**6**), 132.3 (Ar), 130.4 (C**7'**), 129.4 (C**10'**), 128.2 (Ar), 74.0 (C**5**), 69.8 (C**6'**), 66.9 (C**2**), 26.5 (C**4**), 19.2 (C**3**); HRMS (ES^+) calculated for $C_{12}H_{18}N_3O_2$: 236.1399. Found $[M+H]^+$: 236.1403.

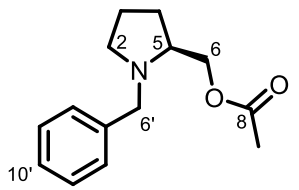
4.4.40. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl methyl carbonate (3.55)



To a stirred solution of *N*-benzyl prolinol (0.50 g, 2.62 mmol, 1.0 eq.) in THF (15 mL) at 0 °C was added pyridine (0.63 mL, 7.84 mmol, 3.0 eq.). The reaction was allowed to stir at this temperature for 20 minutes followed by addition of methyl chloroformate (0.60 mL, 7.84 mmol, 3.0 eq.) dropwise. The solution was allowed to warm to ambient temperature and stirred overnight. Solvents were removed *in vacuo* and the residue was taken up in EtOAc (50 mL). The organic phase was washed with sat. bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.21 g, 32%) as a pale yellow oil.

v max/ cm^{-1} : 1744 (C=O) and 1257 (C-O); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.30 (m, 5H, Ar), 4.17 (dd, 2H, *J* = 10.5, 5.0 Hz, C**6**-CH_2), 4.09 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.80 (s, 3H, C**10**-CH_3), 3.46 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 2.95 (m, 1H, C**2**-H), 2.88 (m, 1H, C**5**-H), 2.28 (m, 1H, C**2**-H), 1.98 (m, 1H, C**3**-H), 1.73 (m, 3H, C**3**-H and C**4**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 155.9 (C**8**), 139.5 (C**7'**), 128.8 (Ar), 128.2 (Ar), 126.9 (C**10'**), 70.6 (C**6**), 61.8 (C**5**), 59.5 (C**6'**), 54.7 (C**10**), 54.4 (C**2**), 28.4 (C**3**), 23.0 (C**4**); HRMS (ES^+) calculated for $C_{14}H_{20}NO_3$: 250.1443. Found $[M+H]^+$: 250.1445.

4.4.41. Preparation of *(S)*-(1-benzylpyrrolidin-2-yl)methyl acetate (3.57)

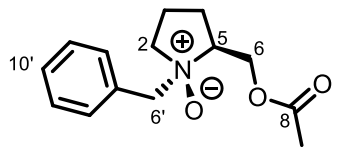


A solution of acetyl chloride (0.22 mL, 3.17 mmol, 1.1 eq.) in DCM (5 mL) was added dropwise to a stirred solution of *N*-benzyl prolinol (0.55 g, 2.88 mmol, 1.0 eq.) and triethylamine (0.44 mL, 3.17 mmol, 1.1 eq.) in DCM (25 mL) at 0 °C. The reaction

was warmed to ambient temperature and stirred for 5 hours. The reaction mixture was diluted with DCM (50 mL) and washed with saturated sodium bicarbonate solution (50 mL), and H_2O (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 1:4 EtOAc:hexanes) to afford the title compound (0.50 g, 75%) as a colourless oil.

v max/ cm^{-1} : 1736 (C=O), 1230 (C-O) and 744 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 (m, 5H, Ar), 4.10 (dd, 1H, *J* = 11.1, 5.3 Hz, C**6**-H), 4.02 (dd, 1H, *J* = 11.0, 6.0 Hz, C**6**-H), 4.08 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.41 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 2.93 (m, 1H, C**2**-H), 2.81 (m, 1H, C**5**-H), 2.25 (m, 1H, C**2**-H), 2.05 (s, 3H, C**9**-CH_3), 1.93 (m, 1H, C**3**-H), 1.69 (m, 3H, C**3**-H and C**4**-CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.1 (C**8**), 139.5 (C**7'**), 128.9 (Ar), 128.2 (Ar), 126.9 (C**10'**), 67.2 (C**6**), 61.8 (C**5**), 59.5 (C**6'**), 54.5 (C**2**), 28.4 (C**3**), 22.9 (C**4**), 21.0 (C**9**); HRMS (ES^+) calculated for $C_{14}H_{20}NO_2$: 234.1494. Found $[M+H]^+$: 234.1488.

4.4.42. Preparation of (1*R*,2*S*)-2-(acetoxymethyl)-1-benzylpyrrolidine 1-oxide (3.58)

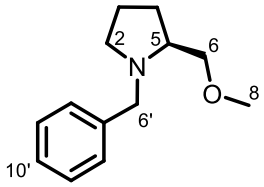


To a stirred solution of tertiary amine **3.57** (0.45 g, 1.93 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.40 g, 2.32 mmol, 1.2 eq.) and K_2CO_3 (0.40 g, 2.99 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.42 g, 88%) as a yellow semi-solid.

v max/ cm^{-1} : 1637 (C=O), 1383 (C-H) and 1002 (N-O); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.65 (m, 2H, Ar), 7.46 (m, 3H, Ar), 4.70 (dd, 2H, *J* = 12.5, 8.0 Hz, C**6**-CH_2), 4.57 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 4.55 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 4.40 (dd, 1H, *J* = 13.0, 3.0 Hz, C**6**-H), 3.96 (m, 1H, C**5**-H), 3.59 (m, 1H, C**2**-H), 3.07 (m, 1H, C**2**-H), 2.15 (s, 3H C**9**-H), 2.14 (m, 2H, C**3**-H, C**4**-H), 1.92 (m, 2H, C**3**-H, C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 170.6 (C**8**), 132.2 (Ar), 130.7 (C**7'**), 129.3 (C**10'**), 128.2 (Ar), 73.5

(C**5**), 69.8 (C**6'**), 66.0 (C**2**), 60.9 (C**6**), 24.8 (C**4**), 19.3 (C**9**), 18.8 (C**3**); HRMS (ES^+) calculated for $C_{14}H_{20}NO_3$: 250.1443. Found $[M+H]^+$: 250.1446.

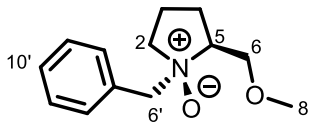
4.4.43. Preparation of *(S)*-1-benzyl-2-(methoxymethyl)pyrrolidine (3.59)



To a stirred solution of *N*-benzyl prolinol (0.50 g, 2.62 mmol, 1.0 eq.) in THF (10 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.21 g, 5.23 mmol, 2.0 eq.) and TBAI (cat.). The reaction was allowed to stir at this temperature for 1 hour followed by the addition of MeI (0.16 mL, 2.62 mmol, 1.0 eq.). The reaction was allowed to warm to ambient temperature and followed by TLC. Upon complete disappearance of starting material the reaction was quenched with methanol and solvents were removed *in vacuo*. The residue was taken up in EtOAc (20 mL) and washed with saturated sodium bicarbonate solution (20 mL) and brine (20 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 7:3 EtOAc:hexanes) to afford the title compound (0.23 g, 43%) as a pale yellow oil.

v max/ cm^{-1} : 1103 (C-O) and 671 (C=C); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.31 (m, 5H, Ar), 4.09 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.43 (dd, 1H, *J* = 9.5, 5.0 Hz, C**6**-H), 3.39 (d, 1H, *J* = 13.0 Hz, C**6'**-H), 3.35 (s, 1H, C**8**-H_3), 3.32 (dd, 1H, *J* = 9.5, 6.0 Hz, C**6**-H), 2.92 (m, 1H, C**2**-H), 2.71 (m, 1H, C**5**-H), 2.20 (m, 1H, C**2**-H), 1.92 (m, 1H, C**4**-H), 1.69 (m, 3H, C**3**-H_2 and C**4**-H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 139.7 (C**7'**), 129.0 (Ar), 128.1 (Ar), 126.8 (C**10'**), 76.5 (C**6**), 63.0 (C**5**), 59.7 (C**6'**), 59.1 (C**8**), 54.6 (C**2**), 28.6 (C**4**), 22.8 (C**3**); HRMS (ES^+) calculated for $C_{13}H_{20}NO$: 206.1545. Found $[M+H]^+$: 206.1552.

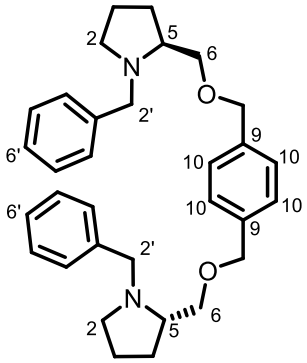
4.4.44. Preparation of (1*R*,2*S*)-1-benzyl-2-(methoxymethyl)pyrrolidine 1-oxide (3.60)



To a stirred solution of tertiary amine **3.59** (0.20 g, 0.97 mmol, 1.0 eq.) in DCM (20 mL) at -78 °C was added *m*-CPBA (0.20 g, 1.17 mmol, 1.2 eq.) and K_2CO_3 (0.20 g, 1.46 mmol, 1.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.21 g, 95%) as a white gum.

v max/ cm^{-1} : 1099 (C-O) and 700 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (m, 2H, Ar), 7.43 (m, 3H, Ar), 4.59 (m, 2H, C**6'-H**), 4.20 (dd, 1H, *J* = 11.0, 8.5 Hz, C**6**-H), 3.83 (m, 1H, C**5**-H), 3.52 (m, 1H, C**6**-H), 3.49 (m, 1H, C**2**-H), 3.46 (s, 3H, C**8**-CH_3), 3.06 (m, 1H, C**2**-H), 2.07 (m, 2H, C**3**-H and C**4**-H), 1.82 (m, 2H, C**4**-H and C**3**-H); ^{13}C NMR (126 MHz, CD_3OD) δ: 132.4 (Ar), 131.1 (C**7'**), 129.1 (C**10'**), 128.1 (Ar), 73.5 (C**5**), 69.9 (C**6'**), 69.2 (C**2**), 65.6 (C**6**), 57.8 (C**8**), 24.3 (C**4**), 18.8 (C**3**); HRMS (ES^+) calculated for $C_{13}H_{20}NO_2$: 222.1494. Found $[M+H]^+$: 222.1493.

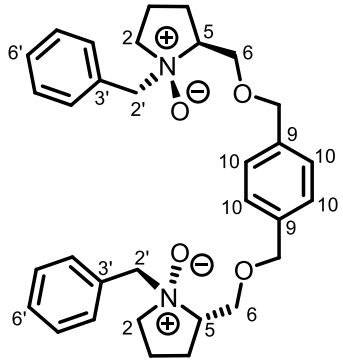
4.4.45. 1,4-bis((((*S*)-1-benzylpyrrolidin-2-yl)methoxy)methyl)benzene (3.65)



To a stirred solution of *N*-benzyl prolinol (0.50 g, 2.62 mmol, 1.0 eq.) in THF (15 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 0.11 g, 2.87 mmol, 1.1 eq.). The reaction was allowed to stir at this temperature for 30 minutes followed by the addition of dibromo-*p*-xylene (0.35 g, 1.31 mmol, 0.5 eq.). The reaction was allowed to warm to ambient temperature and followed by TLC. Upon complete disappearance of starting material the reaction was quenched with methanol and solvents were removed *in vacuo*. The residue was taken up in EtOAc (50 mL) and washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and solvents removed *in vacuo* to give the crude product. The crude material was purified by FCC (eluting with 3:2 EtOAc:hexanes) to afford the title compound (0.29 g, 46%) as a pale yellow oil.

v max/ cm^{-1} : 1083 (C-O) and 698 (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 14H, 14 x Ar), 4.54 (s, 4H, 2 x C**8**-CH_2), 4.15 (d, *J* = 13.0 Hz, 2H, 2 x C**2'**-H), 3.54 (dd, *J* = 9.5, 5.0 Hz, 2H, 2 x C**6**-H), 3.42 (m, 4H, 2 x C**2'**-H and 2 x C**6**-H), 2.94 (m, 2H, 2 x C**2**-H), 2.80 (m, 2H, 2 x C**5**-H), 2.23 (dd, *J* = 16.5, 9.0 Hz, 2H, 2 x C**2**-H), 1.96 (m, 2H, 2 x C**4**-H), 1.70 (m, 6H, 2 x C**4**-H and 2 x C**3**-CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.7 (Ar), 137.8 (Ar), 129.0 (Ar), 128.1 (Ar), 127.7 (Ar), 126.7 (Ar), 74.0 (C**5**), 73.1 (C**6'**), 63.0 (C**2**), 59.7 (C**6**), 54.6 (C**8**), 28.6 (C**4**), 22.8 (C**3**); HRMS (ES^+) calculated for $C_{32}H_{41}N_2O_2$: 415.3168. Found $[M+H]^+$: 415.3170.

4.4.46. Preparation of (1*R*,1'*R*,2*S*,2'*S*)-2,2'-(((1,4-phenylenebis(methylene)) bis(oxy))bis(methylene))bis(1-benzylpyrrolidine 1-oxide) (3.66)



To a stirred solution of bis-amine **3.65** (0.25 g, 0.52 mmol, 1.0 eq.) in DCM (30 mL) at -78 °C was added *m*-CPBA (0.20 g, 1.14 mmol, 2.2 eq.) and K_2CO_3 (0.18 g, 1.29 mmol, 2.5 eq.). The reaction mixture was allowed to slowly warm to ambient temperature and stirred for 18 hours. Solvents were there then removed *in vacuo* and residue was purified by FCC (eluting with 1:1 MeOH:EtOAc) to afford title compound (0.23 g, 87%) as a viscous oil.

v max/ cm^{-1} : 1126 (C-O) and 698 (C=C); ^{1}H NMR (500 MHz, CD_3OD) δ 7.60 (m, 4H, Ar), 7.46 (s, 4H, Ar), 7.37 (m, 6H, Ar), 4.71 (d, 2H, *J* = 11.5 Hz, 2 x C**2'**-H), 4.59 (m, 6H, 2 x C**2'**-H and 2 x C**8**-CH_2), 4.30 (dd, 2H, *J* = 11.0, 8.5 Hz, 2 x C**6**-H), 3.82 (m, 2H, 2 x C**5**-H), 3.64 (dd, 2H, *J* = 11.0, 2.5 Hz, 2 x C**6**-H), 3.48 (m, 2 x C**2**-H), 3.07 (m, 2H, 2 x C**2**-H), 2.09 (m, 2H, 2 x C**3**-H), 2.01 (m, 2H, 2 x C**4**-H), 1.80 (m, 4H, 2 x C**3**-H and 2 x C**4**-H); ^{13}C NMR (126 MHz, CD_3OD) δ 137.6 (Ar), 132.4 (Ar), 131.1 (C**9**), 129.1 (C**6'**), 128.1 (Ar), 127.8 (Ar), 73.2 (C**5**), 72.7 (C**2'**), 69.9 (C**8**), 67.0 (C**6**), 65.6 (C**2**), 24.4 (C**4**), 18.7 (C**3**); HRMS: (ESI^+) Calculated for $C_{32}H_{41}N_2O_4$: 517.3066. Found $[M+H]^+$: 517.3072.

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Appendix A

5. Appendix A

X-Ray crystal data for compound 2.55.1

Table 1 Crystal data and structure refinement for SR655.

Identification code	SR655
Empirical formula	$C_{32}H_{44}N_4O_4$
Formula weight	550.73
Temperature/	100 K
Crystal system	monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 9.5527(15) Å α=90°
	b = 11.3352(18) Å β=96.767(2)°
	c = 13.618(2) Å γ=90°
Volume	1464.3(4) $Å^3$
Z	2
ρ_{calc}	1.249 g/cm^3
μ	0.083 mm^{-1}
F(000)	596.0
Crystal size	0.2 × 0.05 × 0.05 mm^3
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection	3.012 to 65.054°
Index ranges	$-14 \leq h \leq 14$, $-17 \leq k \leq 17$, $-20 \leq l \leq 20$
Reflections collected	50642
Independent reflections	10190 [R_{int} = 0.0292, R_{sigma} = 0.0247]
Data/restraints/parameters	10190/1/361
Goodness-of-fit on F^2	0.972
Final R indexes [I>=2σ (I)]	R_1 = 0.0350, wR_2 = 0.0882
Final R indexes [all data]	R_1 = 0.0411, wR_2 = 0.0927
Largest diff. peak/hole	0.36/-0.19 e $Å^{-3}$
Flack parameter	0.2(2)

Table 2 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for SR655. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O32	10235.7(10)	5141.1(10)	6070.9(7)	18.87(19)
O12	5206.0(10)	5481.9(11)	6608.0(8)	22.5(2)
O21	9060.7(12)	6993.7(10)	2512.2(9)	25.8(2)
N13	3046.2(11)	5071.2(11)	7024.9(8)	15.46(19)
O1	4408.7(16)	7311.2(10)	3828.7(9)	33.9(3)
N4	3664.6(12)	4912.1(10)	3549.4(8)	15.23(19)
N33	8044.8(11)	5064.7(11)	6538.5(9)	17.4(2)
N24	8357.4(12)	5024.0(10)	3628.0(8)	15.52(19)
C11	3994.9(13)	5112.4(12)	6370.5(9)	15.8(2)
C31	8979.2(13)	5433.3(11)	5945.9(9)	15.0(2)
C15	4295.6(13)	4589.2(12)	8677.7(9)	16.3(2)
C34	8457.7(14)	4319.3(12)	7391.4(10)	17.6(2)
C35	9245.4(13)	4959.8(12)	8263.4(9)	16.1(2)
C23	8995.1(14)	6059.1(11)	4141.8(10)	15.7(2)
C3	4172.7(13)	5333.7(11)	4542.7(10)	15.8(2)
C14	3424.7(14)	5477.1(12)	8036.4(10)	16.7(2)
C30	8415.2(14)	6272.9(12)	5126.3(10)	16.4(2)
C10	3486.7(14)	4668.5(12)	5337.5(10)	16.9(2)
C27	8641.6(17)	3869.6(12)	4099.0(11)	21.9(3)
C8	4365.9(14)	5568.6(12)	2813.2(10)	17.5(2)
C18	5888.7(17)	2960.1(16)	9885.5(11)	27.8(3)
C40	10114.7(14)	4318.2(13)	8965.3(10)	19.5(2)
C38	10634.3(16)	6082.4(14)	9937.0(11)	23.0(3)
C16	5678.2(15)	4847.7(14)	9062.2(10)	21.7(3)
C19	4515.4(18)	2691.8(14)	9493.9(11)	25.2(3)
C36	9083.9(17)	6164.4(13)	8408.4(11)	22.9(3)
C37	9768.3(18)	6722.3(14)	9240.2(12)	26.0(3)
C39	10806.1(16)	4876.7(14)	9797.1(11)	22.3(3)
C26	8427.1(17)	5968.5(14)	2035.7(11)	23.9(3)
C22	8623.1(16)	7129.4(12)	3472.7(11)	21.0(3)
C20	3726.4(15)	3497.7(13)	8890.5(11)	20.3(3)
C25	8838.1(15)	4883.3(13)	2650.2(10)	21.1(3)
C28	8187.0(18)	2988.7(13)	3259.2(11)	25.6(3)
C17	6471.6(16)	4037.5(17)	9669.1(11)	27.4(3)
C5	3946.3(19)	3678.4(12)	3324.2(11)	24.9(3)
C7	3878.9(18)	4923.2(14)	1852.3(11)	25.5(3)
C2	3810.9(19)	6647.2(13)	4565.4(12)	25.8(3)
C29	8143.8(19)	3722.7(14)	2297.7(11)	26.6(3)
C9	3968(2)	6853.4(13)	2862.3(12)	27.8(3)
C6	3676(3)	3640.7(15)	2187.4(13)	38.6(5)

Table 3 Anisotropic Displacement Parameters ($Å^2×10^3$) for final. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O32	11.0(4)	25.9(5)	19.3(4)	-0.1(4)	0.1(3)	1.2(3)
O12	12.3(4)	34.8(6)	20.4(5)	-3.6(4)	1.5(3)	-1.9(4)
O21	29.3(5)	24.3(5)	24.6(5)	7.7(4)	6.4(4)	-4.8(4)
N13	12.3(4)	19.3(5)	14.6(4)	-0.5(4)	0.6(3)	-0.4(4)
O1	59.4(8)	17.9(5)	26.5(6)	-4.0(4)	14.2(6)	-13.0(5)
N4	17.4(5)	13.5(5)	14.6(4)	0.3(4)	1.0(4)	-1.3(4)
N33	11.7(4)	22.0(5)	18.3(5)	0.3(4)	0.6(4)	1.0(4)
N24	18.8(5)	13.0(4)	14.4(4)	0.7(4)	0.7(4)	0.3(4)
C11	13.3(5)	18.8(5)	15.2(5)	0.1(4)	1.3(4)	1.6(4)
C31	13.1(5)	15.5(5)	15.7(5)	-3.2(4)	-1.3(4)	-1.0(4)
C15	15.4(5)	21.0(6)	12.7(5)	-1.9(4)	2.3(4)	2.1(4)
C34	18.0(5)	17.1(5)	17.8(6)	-0.3(4)	2.1(4)	-0.6(4)
C35	15.7(5)	17.4(5)	15.8(5)	-0.1(4)	4.2(4)	-0.1(4)
C23	15.0(5)	14.2(5)	17.7(5)	1.7(4)	1.0(4)	-0.7(4)
C3	15.1(5)	17.0(6)	15.3(5)	-2.2(4)	2.0(4)	-1.6(4)
C14	16.5(5)	17.7(5)	15.6(5)	-2.7(4)	0.9(4)	1.2(4)
C30	15.6(5)	15.0(5)	17.9(6)	-1.3(4)	-0.8(4)	0.1(4)
C10	16.5(5)	19.5(6)	14.6(5)	-0.7(4)	1.8(4)	-1.7(4)
C27	33.5(7)	12.3(5)	18.3(6)	0.9(4)	-3.9(5)	1.3(5)
C8	20.0(6)	17.0(5)	15.5(5)	0.6(4)	2.5(4)	-1.5(5)
C18	28.8(7)	38.0(8)	16.4(6)	4.6(6)	1.7(5)	12.5(6)
C40	20.1(6)	16.9(5)	21.1(6)	2.0(5)	1.4(5)	-0.4(5)
C38	24.6(7)	25.8(7)	18.8(6)	-3.7(5)	4.0(5)	-4.5(5)
C16	17.9(6)	31.7(7)	15.5(6)	0.0(5)	1.9(4)	-1.8(5)
C19	32.5(8)	23.0(7)	20.1(6)	1.4(5)	2.6(5)	4.8(6)
C36	29.1(7)	18.2(6)	21.0(6)	-1.3(5)	0.8(5)	6.1(5)
C37	36.4(8)	18.1(6)	23.7(7)	-4.9(5)	4.4(6)	2.1(6)
C39	22.3(6)	25.2(7)	18.8(6)	2.7(5)	-0.6(5)	-1.3(5)
C26	28.4(7)	25.1(7)	18.7(6)	4.7(5)	4.9(5)	0.5(5)
C22	23.9(6)	16.3(6)	22.7(6)	4.1(5)	2.4(5)	-1.9(5)
C20	21.3(6)	20.1(6)	19.3(6)	-1.1(5)	0.6(5)	0.6(5)
C25	23.7(6)	23.2(6)	16.7(6)	0.8(5)	4.1(5)	4.6(5)
C28	37.9(8)	14.5(6)	22.8(7)	-3.4(5)	-2.7(6)	2.3(5)
C17	18.5(6)	47.1(9)	15.8(6)	2.9(6)	-1.3(5)	3.5(6)
C5	42.2(9)	14.0(6)	19.1(6)	-0.7(5)	5.6(6)	-0.5(5)
C7	37.3(8)	22.7(6)	15.7(6)	-0.4(5)	-0.1(5)	-3.7(6)
C2	38.7(8)	16.6(6)	23.8(7)	-3.9(5)	10.5(6)	-3.6(6)
C29	38.2(8)	22.6(7)	18.5(6)	-4.6(5)	0.8(6)	4.9(6)
C9	44.5(9)	16.2(6)	23.8(7)	2.3(5)	9.2(6)	-0.9(6)
C6	76.9(14)	20.1(7)	19.1(7)	-4.3(5)	6.7(8)	-13.9(8)

Table 4 Bond Lengths for SR655.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O32	C31	1.2374(15)	C35	C40	1.3946(18)
O12	C11	1.2371(16)	C35	C36	1.3909(19)
O21	C26	1.430(2)	C23	C30	1.5290(18)
O21	C22	1.4277(19)	C23	C22	1.5336(19)
N13	C11	1.3446(16)	C3	C10	1.5286(18)
N13	C14	1.4568(17)	C3	C2	1.530(2)
O1	C2	1.4262(19)	C27	C28	1.542(2)
O1	C9	1.431(2)	C8	C7	1.523(2)
N4	C3	1.4624(16)	C8	C9	1.509(2)
N4	C8	1.4718(17)	C18	C19	1.390(2)
N4	C5	1.4634(18)	C18	C17	1.388(3)
N33	C31	1.3388(17)	C40	C39	1.394(2)
N33	C34	1.4528(18)	C38	C37	1.388(2)
N24	C23	1.4618(17)	C38	C39	1.392(2)
N24	C27	1.4684(17)	C16	C17	1.398(2)
N24	C25	1.4674(17)	C19	C20	1.390(2)
C11	C10	1.5188(18)	C36	C37	1.391(2)
C31	C30	1.5166(18)	C26	C25	1.513(2)
C15	C14	1.5154(18)	C25	C29	1.525(2)
C15	C16	1.3933(18)	C28	C29	1.548(2)
C15	C20	1.396(2)	C5	C6	1.540(2)
C34	C35	1.5141(19)	C7	C6	1.543(2)

Table 5 Bond Angles for SR655.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C22	O21	C26	110.45(11)	N13	C14	C15	113.16(11)
C11	N13	C14	120.27(11)	C31	C30	C23	114.33(11)
C2	O1	C9	110.89(12)	C11	C10	C3	112.05(11)
C3	N4	C8	109.67(10)	N24	C27	C28	103.40(11)
C3	N4	C5	117.19(11)	N4	C8	C7	102.99(11)
C5	N4	C8	103.34(11)	N4	C8	C9	108.69(11)
C31	N33	C34	121.73(11)	C9	C8	C7	116.65(13)
C23	N24	C27	117.45(10)	C17	C18	C19	119.67(14)
C23	N24	C25	111.36(11)	C35	C40	C39	120.45(13)
C27	N24	C25	103.77(11)	C37	C38	C39	119.33(14)
O12	C11	N13	121.41(12)	C15	C16	C17	120.54(14)
O12	C11	C10	122.77(11)	C18	C19	C20	120.29(15)
N13	C11	C10	115.82(11)	C35	C36	C37	120.83(14)
O32	C31	N33	122.57(12)	C38	C37	C36	120.28(14)
O32	C31	C30	121.60(12)	C38	C39	C40	120.31(14)
N33	C31	C30	115.80(11)	O21	C26	C25	109.95(12)
C16	C15	C14	120.58(12)	O21	C22	C23	112.75(12)
C16	C15	C20	118.92(13)	C19	C20	C15	120.53(13)
C20	C15	C14	120.50(12)	N24	C25	C26	108.96(12)
N33	C34	C35	114.28(11)	N24	C25	C29	102.03(12)
C40	C35	C34	119.22(12)	C26	C25	C29	116.89(13)
C36	C35	C34	121.93(12)	C27	C28	C29	104.86(12)
C36	C35	C40	118.80(13)	C18	C17	C16	120.03(14)
N24	C23	C30	111.92(10)	N4	C5	C6	102.94(12)
N24	C23	C22	107.24(10)	C8	C7	C6	103.58(12)
C30	C23	C22	108.24(11)	O1	C2	C3	112.91(12)
N4	C3	C10	111.74(10)	C25	C29	C28	103.31(12)
N4	C3	C2	106.60(11)	O1	C9	C8	110.01(13)
C10	C3	C2	110.43(11)	C5	C6	C7	105.02(12)

Table 6 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for final.

Atom	*x*	*y*	*z*	U(eq)
H13	2193	4798	6841	19
H33	7157	5280	6408	21
H34A	9061	3674	7190	21
H34B	7601	3957	7605	21
H23	10042	5961	4257	19
H3	5219	5233	4663	19
H14A	2552	5648	8336	20
H14B	3964	6222	8024	20
H30A	8645	7091	5344	20
H30B	7375	6203	5020	20
H10A	2451	4761	5212	20
H10B	3706	3818	5296	20
H27A	8081	3760	4659	26
H27B	9655	3780	4341	26
H8	5411	5486	2968	21
H18	6425	2409	10299	33
H40	10236	3495	8876	23
H38	11105	6463	10504	28
H16	6085	5581	8911	26
H19	4115	1954	9639	30
H36	8499	6612	7934	28
H37	9642	7545	9331	31
H39	11397	4432	10271	27
H26A	8743	5882	1373	29
H26B	7389	6056	1951	29
H22A	7590	7254	3405	25
H22B	9078	7841	3790	25
H20	2792	3304	8621	24
H25	9886	4790	2726	25
H28A	7247	2654	3330	31
H28B	8875	2335	3260	31
H17	7410	4224	9934	33
H5A	3300	3142	3626	30
H5B	4932	3461	3561	30
H7A	4600	4968	1388	31
H7B	2983	5257	1529	31
H2A	2773	6740	4467	31
H2B	4156	6969	5225	31
H29A	8679	3329	1811	32
H29B	7161	3851	1995	32
H9A	4422	7306	2365	33
H9B	2934	6939	2709	33
H6A	2706	3364	1967	46
H6B	4354	3107	1915	46

X-Ray crystal data for compound 3.31

Table 1 Crystal data and structure refinement for SR699.

Identification code	SR699
Empirical formula	$C_{33}H_{46}N_4O_{10}$
Formula weight	658.74
Temperature	100 K
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 11.8919(15) Å α=90°
	b = 16.840(2) Å β=90°
	c = 17.213(2) Å γ=90°
Volume	3447.0(8) $Å^3$
Z	4
ρ_{calc}	1.269 g/cm^3
μ	0.094 mm^{-1}
F(000)	1408.0
Crystal size	0.31 × 0.055 × 0.045 mm^3
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection	4.194 to 52.998°
Index ranges	$-12 \leq h \leq 14$, $-21 \leq k \leq 17$, $-21 \leq l \leq 21$
Reflections collected	23229
Independent reflections	7122 [R_{int} = 0.0377, R_{sigma} = 0.0425]
Data/restraints/parameters	7122/0/435
Goodness-of-fit on F^2	0.990
Final R indexes [I>=2σ (I)]	R_1 = 0.0475, wR_2 = 0.1125
Final R indexes [all data]	R_1 = 0.0636, wR_2 = 0.1227
Largest diff. peak/hole	0.48/-0.36 e $Å^{-3}$
Flack parameter	-0.3(4)

Table 2 Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for SR699. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O10	6373.8(17)	7456.9(13)	5841.5(14)	25.9(5)
O9	4617.4(19)	7982.6(14)	5927.3(15)	29.1(5)
O43	-1219.5(19)	6484.4(13)	5575.9(14)	25.7(5)
O28	-874.5(18)	4543.0(14)	5758.2(14)	28.6(5)
O35	-3572.1(18)	4054.1(14)	6167.7(13)	27.0(5)
O27	858.4(19)	4000.4(14)	5614.0(14)	30.0(6)
O17	8877.1(19)	7740.3(14)	6564.0(14)	29.5(6)
N25	582(2)	5337.8(17)	5800.7(16)	23.1(6)
O46	6686(2)	5540.6(15)	5407.5(16)	34.1(6)
N7	4936(2)	6642.4(17)	5782.1(17)	26.6(6)
N13	8039(2)	7857.4(16)	7112.1(16)	25.0(6)
N31	-2783(2)	3826.1(16)	6721.8(16)	25.1(6)
C26	265(3)	4575(2)	5712.9(18)	22.1(7)
C8	5235(3)	7407(2)	5857.2(19)	23.9(7)
O44	4268(2)	3577(2)	6431.4(19)	60.6(10)
C4	1692(2)	5636.8(19)	5806.2(18)	21.8(7)
C1	3825(3)	6336.2(19)	5797.3(19)	23.5(7)
C2	3685(3)	5542(2)	5618(2)	33.0(8)
C5	1833(3)	6434(2)	6000(2)	25.6(7)
C6	2887(3)	6785(2)	5997(2)	26.0(7)
C37	-3329(3)	4885.9(19)	7671.4(19)	26.8(7)
C29	-1392(3)	3789(2)	5603(2)	28.1(7)
C11	6846(3)	8247(2)	5954(2)	27.4(7)
C15	8587(3)	9172(2)	7465(2)	29.8(8)
C30	-1810(3)	3388.2(19)	6324(2)	27.2(7)
C14	8484(3)	8337(2)	7785(2)	29.8(8)
C12	7123(3)	8408(2)	6786(2)	26.3(7)
C19	8430(3)	6628(2)	7888(2)	31.2(8)
C3	2635(3)	5189(2)	5619(2)	31.0(8)
C23	10215(4)	5971(2)	8055(2)	44.6(10)
C18	7588(3)	7063.9(19)	7387(2)	31.0(8)
C42	-4112(3)	5400(2)	7351(2)	34.2(8)
C36	-2394(3)	4544(2)	7186(2)	28.0(7)
C34	-2319(3)	2580(2)	6142(3)	38.1(9)
C32	-3238(3)	3193(2)	7250(2)	36.6(9)
C22	10008(4)	5871(3)	8830(3)	48.6(11)
C38	-3422(4)	4695(2)	8448(2)	46.0(10)
C33	-3241(4)	2447(2)	6759(3)	44.2(10)
C24	9432(4)	6337(2)	7581(2)	42.1(10)
C41	-4971(3)	5713(3)	7800(2)	43.8(10)
C16	7624(3)	9248(2)	6876(2)	37.5(9)
C40	-5051(4)	5519(2)	8578(3)	43.8(10)
C20	8231(4)	6529(3)	8670(3)	51.2(12)
C39	-4282(4)	5007(3)	8897(3)	54.7(13)

C21	9019(4)	6152(3)	9144(3)	63.7(15)
C45	4075(7)	3110(5)	5716(4)	108(2)
O48	10919(6)	8213(5)	6727(5)	30.3(18)
O49	10969(5)	8457(4)	6391(5)	8.7(14)
O50	10970(8)	8531(5)	6119(6)	38(3)

Table 3 Anisotropic Displacement Parameters ($Å^2×10^3$) for first. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O10	19.2(11)	27.2(12)	31.4(13)	-2.3(10)	-3.8(10)	-1.6(9)
O9	23.9(12)	23.8(12)	39.5(14)	-1.4(11)	-3.7(11)	2.6(10)
O43	25.2(12)	23.8(12)	28.2(12)	-2.5(10)	1.3(10)	0.2(10)
O28	20.6(11)	27.4(13)	37.9(14)	-5.0(11)	7.9(10)	-5.7(10)
O35	20.4(11)	27.5(12)	33.2(13)	-2.3(10)	-0.8(10)	-3.3(9)
O27	27.8(12)	24.8(13)	37.3(14)	2.0(11)	-4.0(11)	0.8(10)
O17	24.4(12)	29.1(13)	35.1(13)	-5.8(11)	-0.4(10)	-0.7(10)
N25	17.0(12)	24.6(15)	27.7(15)	1.0(12)	0.8(11)	2.2(10)
O46	20.7(12)	30.6(14)	51.1(16)	-4.9(12)	-1.6(11)	0.0(10)
N7	17.3(13)	24.3(15)	38.3(17)	-6.7(13)	-2.1(12)	1.7(11)
N13	26.4(14)	23.2(14)	25.4(14)	1.9(12)	-4.1(12)	-1.8(12)
N31	23.2(14)	24.2(14)	27.9(15)	0.9(12)	3.7(12)	-1.9(11)
C26	23.0(15)	25.3(18)	18.0(16)	2.7(13)	0.0(13)	0.4(14)
C8	22.3(15)	31.0(18)	18.5(16)	0.2(14)	-3.0(13)	-0.9(14)
O44	29.5(15)	100(3)	51.7(19)	4.2(19)	1.3(14)	-19.8(17)
C4	18.8(15)	26.8(17)	19.9(15)	2.1(13)	-3.0(13)	-1.2(13)
C1	18.4(15)	26.5(18)	25.4(17)	-0.9(14)	-4.5(13)	0.0(13)
C2	18.2(16)	26.9(19)	54(2)	-7.0(17)	-0.3(15)	5.3(14)
C5	20.7(16)	27.1(18)	29.0(17)	-5.6(14)	2.5(13)	3.3(13)
C6	23.3(16)	24.4(17)	30.3(18)	-6.0(14)	1.1(14)	0.6(13)
C37	29.4(17)	22.7(17)	28.2(18)	-3.5(14)	3.5(15)	-6.3(14)
C29	23.5(16)	26.7(18)	34.2(19)	-6.2(15)	5.7(14)	-4.3(14)
C11	22.4(16)	27.9(18)	31.9(18)	3.2(15)	-3.7(14)	-8.3(14)
C15	34.8(19)	23.6(18)	31.1(18)	-4.0(15)	-4.7(15)	-3.1(15)
C30	26.0(17)	21.4(17)	34.1(19)	-1.9(14)	1.8(15)	1.2(14)
C14	34.4(19)	24.8(18)	30.1(18)	-4.4(14)	-7.8(15)	-2.7(15)
C12	24.5(16)	24.7(18)	29.7(18)	0.0(14)	-4.1(14)	1.3(14)
C19	40(2)	20.1(17)	33.6(19)	4.3(15)	-5.9(16)	-7.6(15)
C3	21.5(16)	21.2(17)	50(2)	-3.6(16)	-1.7(16)	2.2(14)
C23	54(2)	32(2)	48(2)	-0.6(18)	-5(2)	16(2)
C18	35.6(19)	21.5(17)	36(2)	3.7(15)	-7.0(16)	-6.0(15)
C42	39(2)	34(2)	29.9(19)	-3.1(16)	4.5(16)	5.7(17)
C36	26.9(17)	26.0(17)	31.0(19)	-0.9(15)	1.0(14)	-4.4(14)
C34	38(2)	21.2(18)	55(3)	-4.0(17)	7.0(19)	-3.4(16)
C32	40(2)	28(2)	41(2)	8.4(16)	11.0(18)	-5.9(16)
C22	46(2)	45(2)	55(3)	24(2)	-6(2)	6(2)
C38	65(3)	41(2)	32(2)	0.8(18)	8(2)	10(2)
C33	45(2)	24.5(19)	63(3)	2.8(19)	14(2)	-8.8(18)
C24	60(3)	32(2)	35(2)	-8.6(17)	-6.0(19)	17.1(18)
C41	37(2)	47(3)	47(2)	-11(2)	5.4(19)	9.5(18)
C16	46(2)	21.6(18)	45(2)	2.8(16)	-14.2(18)	-1.7(17)
C40	47(2)	34(2)	50(3)	-13(2)	22(2)	-4.2(19)
C20	36(2)	66(3)	52(3)	30(2)	4.0(19)	2(2)
C39	87(3)	40(2)	37(2)	-2(2)	27(2)	4(2)
C21	47(3)	97(4)	47(3)	45(3)	5(2)	6(3)

Table 4 Bond Lengths for SR699.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O10	C8	1.358(4)	C5	C6	1.386(5)
O10	C11	1.458(4)	C37	C42	1.386(5)
O9	C8	1.222(4)	C37	C36	1.505(5)
O28	C26	1.358(4)	C37	C38	1.379(5)
O28	C29	1.436(4)	C29	C30	1.499(5)
O35	N31	1.392(4)	C11	C12	1.494(5)
O27	C26	1.210(4)	C15	C14	1.516(5)
O17	N13	1.387(4)	C15	C16	1.535(5)
N25	C26	1.347(4)	C30	C34	1.523(5)
N25	C4	1.413(4)	C12	C16	1.542(5)
N7	C8	1.342(4)	C19	C18	1.512(5)
N7	C1	1.418(4)	C19	C24	1.392(6)
N13	C14	1.508(4)	C19	C20	1.377(6)
N13	C12	1.537(4)	C23	C22	1.367(6)
N13	C18	1.515(4)	C23	C24	1.383(6)
N31	C30	1.533(4)	C42	C41	1.384(5)
N31	C36	1.522(4)	C34	C33	1.541(5)
N31	C32	1.502(4)	C32	C33	1.513(6)
O44	C45	1.480(8)	C22	C21	1.379(6)
C4	C5	1.393(5)	C38	C39	1.385(6)
C4	C3	1.390(4)	C41	C40	1.382(6)
C1	C2	1.383(5)	C40	C39	1.371(7)
C1	C6	1.390(4)	C20	C21	1.394(6)
C2	C3	1.382(5)			

Table 5 Bond Angles for SR699.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C8	O10	C11	116.0(3)	C38	C37	C42	118.5(3)
C26	O28	C29	116.9(3)	C38	C37	C36	120.5(3)
C26	N25	C4	127.0(3)	O28	C29	C30	112.7(3)
C8	N7	C1	126.4(3)	O10	C11	C12	112.2(3)
O17	N13	C14	110.3(2)	C14	C15	C16	104.9(3)
O17	N13	C12	110.3(2)	C29	C30	N31	113.8(3)
O17	N13	C18	109.9(2)	C29	C30	C34	111.4(3)
C14	N13	C12	101.9(2)	C34	C30	N31	102.8(3)
C14	N13	C18	110.9(3)	N13	C14	C15	104.2(3)
C18	N13	C12	113.3(3)	N13	C12	C16	104.0(3)
O35	N31	C30	109.6(2)	C11	C12	N13	113.4(3)
O35	N31	C36	110.2(2)	C11	C12	C16	110.3(3)
O35	N31	C32	111.6(3)	C24	C19	C18	121.4(3)
C36	N31	C30	112.8(2)	C20	C19	C18	120.2(4)
C32	N31	C30	101.5(3)	C20	C19	C24	118.4(4)
C32	N31	C36	110.9(3)	C2	C3	C4	119.7(3)
O27	C26	O28	123.9(3)	C22	C23	C24	120.5(4)
O27	C26	N25	128.0(3)	C19	C18	N13	111.8(3)
N25	C26	O28	108.1(3)	C41	C42	C37	120.7(4)
O9	C8	O10	123.5(3)	C37	C36	N31	111.8(3)
O9	C8	N7	127.7(3)	C30	C34	C33	105.7(3)
N7	C8	O10	108.8(3)	N31	C32	C33	104.6(3)
C5	C4	N25	117.2(3)	C23	C22	C21	119.6(4)
C3	C4	N25	124.0(3)	C37	C38	C39	120.8(4)
C3	C4	C5	118.8(3)	C32	C33	C34	105.3(3)
C2	C1	N7	117.4(3)	C23	C24	C19	120.7(4)
C2	C1	C6	118.9(3)	C40	C41	C42	120.2(4)
C6	C1	N7	123.7(3)	C15	C16	C12	106.2(3)
C1	C2	C3	121.6(3)	C39	C40	C41	119.3(4)
C6	C5	C4	121.2(3)	C19	C20	C21	120.8(4)
C5	C6	C1	119.7(3)	C40	C39	C38	120.5(4)
C42	C37	C36	121.0(3)	C22	C21	C20	120.0(4)

Table 6 Hydrogen Atom Coordinates (Å×10⁴) and Isotropic Displacement Parameters (Å²×10³) for SR699.

Atom	*x*	*y*	*z*	U(eq)
H43A	-1274	6877	5884	39
H43B	-1101	6651	5118	39
H25	39	5688	5861	28
H46A	7352	5743	5475	51
H46B	6719	5079	5633	51
H7	5483	6297	5717	32
H44	4879	3825	6393	91
H2	4325	5231	5491	40
H5	1195	6742	6138	31
H6	2969	7329	6130	31
H29A	-839	3439	5343	34
H29B	-2030	3868	5242	34
H11A	6300	8649	5769	33
H11B	7537	8300	5638	33
H15A	9324	9249	7208	36
H15B	8507	9570	7885	36
H30	-1175	3323	6700	33
H14A	9225	8134	7957	36
H14B	7956	8321	8230	36
H12	6427	8361	7109	32
H3	2559	4643	5491	37
H23	10903	5787	7839	53
H18A	6889	7151	7687	37
H18B	7400	6732	6930	37
H42	-4059	5539	6817	41
H36A	-1765	4386	7530	34
H36B	-2112	4956	6825	34
H34A	-1740	2159	6175	46
H34B	-2647	2576	5614	46
H32A	-4008	3326	7424	44
H32B	-2751	3126	7711	44
H22	10543	5610	9150	58
H38	-2890	4346	8678	55
H33A	-3983	2370	6509	53
H33B	-3068	1975	7080	53
H24	9579	6391	7041	51
H41	-5506	6061	7572	53
H16A	7911	9443	6371	45
H16B	7046	9623	7068	45
H40	-5634	5738	8889	53
H20	7549	6719	8889	61
H39	-4340	4865	9429	66
H21	8873	6089	9683	76
H45A	4457	3366	5278	162
H45B	3266	3081	5610	162

X-Ray crystal data for compound 2.29

Table 1 Crystal data and structure refinement for SR352.

Identification code	SR352
Empirical formula	$C_9H_{15}NO_3$
Formula weight	185.22
Temperature	150.0 K
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 6.0424(4) Å α=90°
	b = 10.3964(7) Å β=90°
	c = 14.5053(10) Å γ=90°
Volume/	911.21(11) $Å^3$
Z	4
ρ_{calc}	1.350 g/cm^3
μ/mm^{-1}	0.836
F(000)	400.0
Crystal size/	0.221 × 0.03 × 0.03 mm^3
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection	10.46 to 133.28°
Index ranges	$-6 \leq h \leq 7$, $-12 \leq k \leq 12$, $-17 \leq l \leq 17$
Reflections collected	9203
Independent reflections	1608 [R_{int} = 0.0331, R_{sigma} = 0.0229]
Data/restraints/parameters	1608/0/119
Goodness-of-fit on F^2	1.096
Final R indexes [I>=2σ (I)]	R_1 = 0.0289, wR_2 = 0.0718
Final R indexes [all data]	R_1 = 0.0307, wR_2 = 0.0731
Largest diff. peak/hole	0.15/-0.21 e $Å^{-3}$
Flack parameter	0.1(2)

Table 2 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for SR352. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O6	3848.4(18)	2436.1(8)	7167.0(7)	21.4(2)
O8	1913(2)	5913.0(11)	6164.9(7)	30.8(3)
O13	6191(2)	3365.9(11)	4168.4(7)	29.0(3)
N1	3822(2)	3776.4(10)	7148.1(8)	17.4(3)
C11	6152(3)	3564.5(13)	5795.1(10)	21.6(3)
C10	4829(2)	4308.5(14)	6286.0(9)	19.4(3)
C4	1097(3)	3944.1(15)	8320.2(10)	24.6(3)
C2	5036(2)	4256.9(14)	7993.2(9)	21.8(3)
C12	7307(3)	3942.4(14)	4924.6(11)	24.7(3)
C7	1192(3)	5640.3(14)	7075.3(10)	27.5(3)
C5	1452(2)	4217.4(14)	7301.7(9)	21.1(3)
C9	4239(3)	5695.5(15)	6101.8(10)	26.2(4)
C3	3359(3)	4145.6(16)	8777.3(10)	28.1(4)

Table 3 Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for SR352. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O6	28.2(6)	14.4(5)	21.7(5)	-0.9(4)	-1.0(5)	1.2(4)
O8	37.3(7)	30.8(6)	24.2(5)	3.6(4)	-0.4(5)	14.6(5)
O13	30.9(6)	35.1(6)	21.1(5)	-5.1(4)	3.2(5)	3.2(5)
N1	18.7(6)	16.3(5)	17.3(5)	-0.8(4)	-2.1(5)	0.7(5)
C11	23.4(8)	20.4(7)	21.1(7)	-0.6(6)	-2.9(6)	-0.5(6)
C10	22.4(8)	19.9(7)	15.8(6)	-1.1(6)	-2.3(6)	-1.4(6)
C4	25.5(8)	23.3(7)	25.1(7)	-2.8(6)	3.8(6)	-2.1(7)
C2	23.3(8)	24.9(7)	17.1(7)	-2.1(6)	-4.6(6)	-2.6(6)
C12	26.7(8)	24.3(7)	23.1(7)	-2.6(6)	3.7(6)	0.8(6)
C7	29.2(8)	27.1(7)	26.4(7)	0.0(6)	2.5(7)	9.4(7)
C5	16.5(8)	23.2(7)	23.5(7)	-3.7(6)	-1.3(5)	1.3(6)
C9	35.6(10)	21.9(8)	21.2(7)	1.3(6)	3.5(6)	2.2(7)
C3	30.2(9)	34.7(9)	19.4(7)	-4.2(6)	1.1(6)	-0.7(7)

Table 4 Bond Lengths for SR352

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O6	N1	1.3938(13)	C11	C10	1.321(2)
O8	C7	1.4192(18)	C11	C12	1.495(2)
O8	C9	1.427(2)	C10	C9	1.509(2)
O13	C12	1.4202(18)	C4	C5	1.5196(19)
N1	C10	1.4965(18)	C4	C3	1.534(2)
N1	C2	1.5134(17)	C2	C3	1.527(2)
N1	C5	1.5202(19)	C7	C5	1.523(2)

Table 5 Bond Angles for SR352

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C7	O8	C9	109.30(12)	C11	C10	C9	127.37(13)
O6	N1	C10	112.42(10)	C5	C4	C3	105.60(13)
O6	N1	C2	107.97(10)	N1	C2	C3	104.88(11)
O6	N1	C5	108.02(10)	O13	C12	C11	108.65(12)
C10	N1	C2	110.97(10)	O8	C7	C5	111.28(12)
C10	N1	C5	113.20(10)	N1	C5	C7	111.01(12)
C2	N1	C5	103.78(10)	C4	C5	N1	102.66(12)
C10	C11	C12	125.73(13)	C4	C5	C7	112.12(12)
N1	C10	C9	113.90(11)	O8	C9	C10	111.88(13)
C11	C10	N1	118.69(12)	C2	C3	C4	106.23(11)

Table 6 Torsion Angles for SR352.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
O6	N1	C10	C11	-19.43(18)	C2	N1	C10	C11	101.61(15)
O6	N1	C10	C9	162.69(12)	C2	N1	C10	C9	-76.27(15)
O6	N1	C2	C3	-79.84(14)	C2	N1	C5	C4	-41.51(13)
O6	N1	C5	C4	72.93(12)	C2	N1	C5	C7	78.47(13)
O6	N1	C5	C7	-167.08(10)	C12	C11	C10	N1	-179.45(14)
O8	C7	C5	N1	55.28(16)	C12	C11	C10	C9	-1.9(3)
O8	C7	C5	C4	169.45(13)	C7	O8	C9	C10	62.36(16)
N1	C10	C9	O8	-49.90(16)	C5	N1	C10	C11	-142.17(13)
N1	C2	C3	C4	-14.34(16)	C5	N1	C10	C9	39.95(15)
C11	C10	C9	O8	132.44(16)	C5	N1	C2	C3	34.64(14)
C10	N1	C2	C3	156.53(12)	C5	C4	C3	C2	-11.30(16)
C10	N1	C5	C4	-161.91(11)	C9	O8	C7	C5	-65.71(17)
C10	N1	C5	C7	-41.93(14)	C3	C4	C5	N1	32.33(14)
C10	C11	C12	O13	-104.25(17)	C3	C4	C5	C7	-86.88(16)

Table 7 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for SR352

Atom	*x*	*y*	*z*	U(eq)
H13	7106	3201	3749	44
H11	6391	2715	6014	26
H4A	577	3050	8413	30
H4B	-13	4541	8583	30
H2A	5510	5161	7910	26
H2B	6357	3722	8119	26
H12A	7297	4890	4858	30
H12B	8865	3649	4942	30
H7A	-382	5889	7140	33
H7B	2066	6157	7518	33
H5	411	3692	6921	25
H9A	5012	6251	6553	31
H9B	4756	5937	5478	31
H3A	3355	4940	9154	34
H3B	3727	3408	9181	34